The role of earthworms for plant performance and ecosystem functioning in a plant diversity gradient

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“Earthworms are the intestines of the soil”

Aristotle (about 330 B.C.)

THE JENA EXPERIMENT

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe. Ich habe noch keinen weiteren Promotionsversuch unternommen. Die Versuche in Kapitel 2, 3 und 4 wurden in Zusammenarbeit mit Stephan Partsch durchgeführt. Die in dieser Arbeit dargestellten Ergebnisse basieren auf den von mir in diesen Versuchen erhobenen Daten.

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Publications

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Contents

Zusammenfassung

Im Rahmen des „Jena Biodiversitäts Experiments“ wurde der Einfluss von Regenwürmern auf Pflanzen und Ökosystemprozesse in einem Gradienten zunehmender Pflanzendiversität untersucht. Außerdem wurde der Einfluss der Pflanzendiversität auf Regenwürmer, Springschwänze, Mikroorganismen und Zersetzungsprozesse untersucht. Dazu wurden ein Freilandversuch und zwei Gewächshausversuche durchgeführt.

In dem ersten Labor-Mikrokosmosversuch wurde der Einfluss der Diversität von Pflanzen (1, 2, 4, 8 Arten), von funktionellen Pflanzengrupen (1, 2, 3, 4 funktionelle Gruppen) sowie der Identität der Pflanzengruppen (Gräser, Leguminosen, große und kleine Kräuter) auf drei Zersetzergruppen (Regenwürmer, Springschwänze, Mikroorganismen) untersucht. Weiterhin wurde die Reaktion der Pflanzengemeinschaft auf die Präsenz von zwei tierischen Zersetzergruppen (Regenwürmer, Springschwänze) untersucht. Für diesen Versuch wurde ^{15}N markierte Streu benutzt, um die Nährstoffflüsse nachvollziehen zu können.

Folgende Hypothesen wurden geprüft:

(1) Regenwürmer, Mikroorganismen und Zersetzungsprozesse profitieren von bzw. werden beschleunigt durch zunehmende Diversität von Pflanzenarten und funktionellen Pflanzengruppen.

(2) Die Wirkung von Regenwürmern und Springschwänzen in der Rhizosphäre auf die Artenzusammensetzung und Produktivität von Pflanzengemeinschaften hängt von der Pflanzendiversität ab.

Entsprechend Hypothese (1) nahm die Biomasse und ^{15}N-Aufnahme von Regenwürmern mit zunehmender Pflanzendiversität und mit der Zunahme funktioneller Pflanzengruppen zu. Vor allem die Anwesenheit von Leguminosen beeinflusste die Regenwurmbiomasse stark positiv. Die Effekte zunehmender Pflanzendiversität wirkten sich auf die verschiedenen funktionellen Gruppen der Regenwürmer unterschiedlich aus. Die anözische Regenwurmart *Lumbricus terrestris* wurde vor allem von der Präsenz stickstoffreicher Leguminosen positiv beeinflusst. Die endogäischen Arten profitierten ebenfalls von der zunehmenden Diversität von Pflanzen, jedoch verursacht durch unterirdische Effekte, vermutlich basierend auf Wurzelexsudaten.

Eine zunehmende Diversität von Pflanzenarten sowie von funktionellen Pflanzengruppen beeinflusste auch Mikroorganismen im Boden. Die mikrobielle Respiration nahm mit zunehmender Diversität von Pflanzenarten und von funktionellen Pflanzengruppen zu. Die Respirationsraten der Mikroorganismen waren mit der Wurzelbiomasse korreliert.

Leguminosen erhöhten die mikrobielle Respiration, während die Präsenz von Gräsern sie reduzierte. Generell reagierte die mikrobielle Biomasse jedoch nur wenig auf Änderungen der Pflanzendiversität.

Entsprechend Hypothese (2) wurde der ^{14}N- und ^{15}N-Gehalt von Pflanzen durch die Anwesenheit verschiedener Zersetzergruppen signifikant beeinflusst. Mit zunehmender Diversität von Pflanzenarten und funktionellen Pflanzengruppen nahmen die Sprossbiomasse und die Anzahl und das Gewicht von Pflanzensamen zu. Die Sprossbiomasse nahm sogar dann mit zunehmender Pflanzendiversität zu, wenn die Biomasse der Leguminosen unberüchsicktigt blieb. Zunehmende Diversität von funktionellen Pflanzengruppen, jedoch nicht die Zunahme der Artendiversität, führte zu einer Zunahme der gesamten Pflanzenbiomasse. Die Präsenz der Zersetzer führte zu einer Zunahme der Sprossbiomasse, mit einem Maximum in den Varianten mit Regenwürmern. Die Wurzelbiomasse nahm in Anwesenheit von Collembolen und Regenwürmern ab. In Varianten mit Zersetzern nahmen der Stickstoff-Gehalt des Pflanzengewebes und die ^{15}N-Gehalte zu.

In dem zweiten Gewächshaus-Mikrokosmosversuch wurde der Einfluss der anözischen Regenwurmart *L. terrestris* auf die Keimungsrate und die Aggregation von Pflanzensamen untersucht indem die Anzahl von funktionellen Pflanzengruppen (Gräser, Leguminosen, Kräuter), die Samengröße (klein und groß), die Artendiversität der Samen (1, 3, 6) und deren Zugehörigkeit zu funktionellen Pflanzengruppen (1, 3) variiert wurde. *L. terrestris* beeinflusste die Keimungsrate in Abhängigkeit von der Samengröße und der Zugehörigkeit der Samen zu funktionellen Pflanzengruppen. Zusätzlich führten Regenwürmer zu heterogeneren Mikrohabitaten und förderten dadurch eine diversere Pflanzengemeinschaft.

In dem Freilandexperiment wurde eine ähnliche Fragestellung wie in dem ersten Gewächshausexperiment untersucht. Die Siedlungsdichte von Regenwürmern (erhöht und reduziert) und der Springschwänze (reduziert und unbeeinflusst) wurde verändert. Außerdem wurde die Streuabbaurate in unterschiedlich diversen Pflanzengemeinschaften (1, 4, 16 Arten bzw. 1, 2, 3 funktionelle Pflanzengruppen) untersucht.

Wie in dem Mikrokosmos-Experiment stieg mit zunehmender Pflanzendiversität die Biomasse der Regenwürmer. Die mikrobielle Respirationsrate nahm bei erhöhter Pflanzendiversität zu und korrelierte mit der Wurzelbiomasse. Die Bodenbeschaffenheit und das Vorhandensein von Leguminosen, jedoch nicht die Pflanzendiversität, veränderte die Regenwurmgemeinschaft. Die Zersetzungsrate der Streu wurde hauptsächlich durch lokale

abiotische Faktoren (Block-Effekt) und weniger durch Pflanzendiversität beeinflusst. Wie erwartet hing die Streuabbaurate von der Streuart ab; Leguminosenstreu wurde schneller abgebaut als Gras- und Kräuterstreu. Regenwürmer und eine erhöhte Diversität funktioneller Pflanzengruppen beschleunigten die Zersetzung und Mineralisierung von stickstoffreicher Leguminosenstreu. Vermutlich tragen Regenwürmer vor allem in diversen Pflanzengemeinschaften zu erhöhter Produktivität bei. Der Einfluss der Regenwürmer auf die Pflanzen war im Gewächshausexperiment deutlicher als im Feldversuch. Zwei Jahre nach der Etablierung der Pflanzen im Freiland konnten keine signifikanten Zusammenhänge zwischen Regenwurmdichte und Pflanzenbiomasse oder Pflanzenzusammensetzung festgestellt werden. Insgesamt zeigen die Ergebnisse eine starke gegenseitige Abhängigkeit von Pflanzendiversität und Zersetzeraktivität. Vor allem Leguminosen sind eine wichtige funktionelle Pflanzengruppe, die die Zersetzer im Boden stark positiv beeinflussen. Tierische Zersetzer fördern generell das Pflanzenwachstum und manche Zersetzer, wie z.B. anözische Regenwürmer, erhöhen auch die pflanzliche Diversität.

Summary

The role of earthworms for plant performance and ecosystem functioning in a plant diversity gradient was investigated as part of the "Jena Biodiversity Experiment". Concomitantly effects of the plant diversity on earthworms, springtails, microorganisms and decomposition were studied. Two greenhouse experiments and one field experiment were conducted.

The first greenhouse experiment focused on the responses of three decomposer groups (earthworms, springtails and microorganisms) to manipulations in plant species diversity (1, 2, 4, 8), plant functional group diversity (1, 2, 3, 4) and functional group identity (grasses, legumes, small herbs, tall herbs) in a microcosm experiment. Also, the response of the plant community to the four decomposer treatments (control, earthworms, springtails and combined) was investigated. The use of ^{15}N labelled litter allowed tracking of nutrient fluxes from dead organic matter into plants and animals.

We hypothesised (1) that an increase in plant species and functional group diversity will beneficially affect earthworms and microorganisms, and accelerate decomposition processes, (2) that plant species and functional groups will differentially respond to earthworms and springtails in the rhizosphere.

As hypothesised, earthworm performance (biomass and ^{15}N incorporation) increased with increasing plant species and functional group diversity. Presence of legumes also beneficially affected earthworm performance. The mechanism of the beneficial effect of increasing plant species diversity differed between the earthworm functional groups. For anecic species (*Lumbricus terrestris*) the effect of plant diversity was mainly due to the presence of legumes; nitrogen rich legume litter being preferred by this species. The endogeic species also benefited from increased plant diversity, but via belowground effects of plant diversity based on rhizodeposits. Increasing plant species and functional group diversity also affected microorganisms in soil. Respiration rates decreased with increasing plant species and functional group diversity, but correlated with root biomass. Identity of plant functional groups was also important; legumes increased and grasses decreased microbial respiration. Microbial biomass, however, was little responsive to changes in plant diversity.

Plant performance (biomass, N tissue concentration, ^{15}N) was strongly affected by the decomposer treatments and plant diversity. Increasing plant species and plant functional group diversity increased total number and total weight of seeds. Shoot biomass increased

with increasing plant species diversity and, even more pronounced, with plant functional group diversity. The increased shoot biomass with increasing plant species and functional group diversity remained significant when calculated without legume biomass. Increasing plant species diversity but not plant functional group diversity, decreased root biomass. Plant functional group diversity but not plant species diversity, increased total plant biomass. Plant functional group identity mattered; grasses benefited most from the presence of earthworms. Decomposers strongly increased shoot biomass, being at a maximum in the earthworm only treatment. Root biomass decreased in presence of collembolans, and even stronger in presence of earthworms; however, it increased when both animal groups were present. In treatments with decomposers, total N tissue concentration and ^{15}N enrichment of three focal species was increased.

In the second greenhouse experiment the effect of the anecic earthworm *L. terrestris* on plant seedling recruitment and aggregation was investigated by varying the number of plant functional groups (grass, legumes, herbs), seed size (small and large), plant species diversity (1, 3, 6) and plant functional group diversity (1, 3). *L. terrestris* strongly affected the recruitment of plant seedlings depending on seed size and plant functional group. Furthermore, earthworms increased microhabitat heterogeneity. Seed translocation, surface cast deposition and formation of burrows presumably act as intermediate disturbances favouring the formation of a more diverse plant community.

In the field experiment similar hypothesis as in the first greenhouse experiment were investigated. Manipulations of the density of earthworms (reduced and increased) and springtails (reduced and natural) were established. In addition, decomposition of litter as affected by plant species (1, 4, 16) and functional group diversity (1, 2, 3), decomposers and litter functional group identity was investigated.

Consistent with the microcosm experiment earthworm performance (biomass) was increased with increasing plant species diversity. Microbial respiration increased with increasing plant species diversity and was correlated with root biomass. Soil texture and presence of legumes but not plant diversity affected the community composition of earthworms. Decomposition of litter was primarily affected by local abiotic conditions (block effects) and less by the plant diversity gradient. As expected, litter decomposition was strongly affected by the identity of plant functional groups; legume litter decomposed faster than grass and herb litter. Earthworms and increasing plant functional group diversity increased the decomposition of

legume litter. The increase in earthworm density with increasing plant diversity accelerated the decomposition and mineralization of nitrogen rich organic matter; therefore, earthworms may have contributed to higher plant productivity in particular in treatments with high plant species and functional group diversity.

The effects of earthworms on plant performance were more distinct in the greenhouse experiments than in the field. After two years from the establishment of the plant communities in the field we did not find significant effects of earthworms on plant performance (biomass) or plant community composition (plant species diversity or cover).

Overall, the results document that there is a strong interdependence between plant diversity and the functioning of the decomposers and vice versa. The results suggest that increasing plant diversity has beneficial effects on decomposer performance. Legumes represent a key plant functional group that strongly affects the decomposition processes at least in part via beneficial effects on soil decomposer invertebrates, such as earthworms. The results also show that plant performance is beneficially affected by decomposers and that some decomposers, such as anecic earthworms, are promoting plant diversity.

Chapter 1

General Introduction

1.1 Biodiversity and ecosystem functioning

The extent to which ecosystem functioning depends on biodiversity has risen as a crucial question at a time when human activities accelerated the rate at which species are disappearing (Ehrlich 1988, Soule 1991). Earth's biota with its extraordinary diversity estimated around 10 million species suffers such high extinction rates that even at the lowest estimated rates about half of the species are expected to go extinct within the next 100 years (Soule 1991, Chapin et al. 2001). Understanding the functioning of ecosystems requires not only knowledge of biogeochemical processes, but also of the role of biodiversity. It became critical to understand how the loss, or addition, of species influences the stability and functioning of ecosystems we rely on.
Three main patterns of biodiversity – ecosystem functioning relationships have been formulated (Loreau 2002, Hooper et al. 2005):

(1) *Species are primarily redundant – the redundancy hypothesis*. This implies that the loss of species is compensated by other species or the addition of species adds little new to the functioning of the system. A variation of the redundancy hypothesis is *the rivet hypothesis* (Ehrlich & Ehrlich 1981) which states that the redundancy is important to a point where so many species are lost that the system fails.

(2) *Species are primarily singular.* This hypothesis implies that the contribution of each species to ecosystem functioning is unique and that loss or addition of species cause detectable changes in functioning. Increasing ecosystem functioning with increasing diversity may arise from two mechanisms:
(i) Sampling effect (selection probability effect) – increasing species richness increases the probability that key species are present (Aarssen 1997, Huston 1997, Loreau 2000).
(ii) Species richness or functional richness – increase in ecosystem functioning through positive interactions between species, with complementarity and facilitation as the two main

mechanisms leading to e.g. overyielding in grassland communities (Harper 1977, Loreau 1998, Petchey 2003). In overyielding plant production in mixtures exceeds expectations based on monoculture yields. Complementarity results from reducing the interspecific competition through niche partitioning (Harper 1977, Ewel 1986, Vandermeer 1989). Facilitation can occur if certain species provide a critical resource for other species or improves environmental conditions (Fowler 1986, Mulder et al. 2001, Bruno et al. 2003).

(3) *A saturating response of ecosystem properties to increasing species richness.* This is the most commonly hypothesised theory assuming that the more depauperated a community becomes the stronger the effect of extinction on ecosystem functioning, due to the loss of complementarity and facilitation will be. Changes might be idiosyncratic (sensu Lawton 1994, Naem et al. 1995) and determinated by the traits of the species going extinct or remaining in the community.
In the last years two early stand alone hypothesis: '*the keystone hypothesis'* (i.e. species whose loss has a disproportionate impact on the ecosystem functioning when compared to the loss of other species) and '*the idiosyncratic hypothesis*' (i.e. unpredictable impact of species loss or addition on ecosystem functioning) have been assimilated by hypothesis (2) and (3).

1.2 Decomposer – producer interactions and ecosystem functioning

The concern that loss of biodiversity can affect the ability of ecosystems to provide services to mankind has lead in the last years to a number of experimental studies aiming to investigate the effect of biodiversity on ecosystem functioning (reviews by Schäpfer & Schmidt 1999, Loreau et al. 2002, Hooper et al. 2005). The experimental approach of most of these studies was to use experimental plant assemblages of varying species and functional group diversity and to concentrate on aboveground biomass as an indicator of net primary production (Naem et al. 1994, 1996, Tilman et al. 1996, Hector et al. 1999, Tilman 1999). There has been considerably less work on other ecosystem processes which may not respond in the same way as productivity.
In particular, decomposition plays a critical role for mineralizing nutrients that enter the decomposer subsystem as litter and which in turn feedback to primary production. In this way the belowground compartment of ecosystems is intimately linked with the aboveground

compartment (Swift et al. 1979, Schlesinger 1997). The interrelationships between above- and belowground compartments are stronger then previously assumed and the direct and indirect interactions between them have the potential to operate as major drivers of ecosystem processes (Hooper et al. 2000, Van der Putten et al. 2001, Wardle 2002).

Plant diversity effects on decomposers

Plant species or functional group diversity influence decomposition processes by altering the quality and quantity of the resources entering the soil, the amount of soluble carbon compounds liberated via root exudates (Li et al. 2004, Bais et al. 2004), or competition for nutrients (Okano et al. 1991, Grime 1994, Fransen et al. 1999).

The studies that investigated effects of increasing species richness on soil organisms and processes reported positive responses (Hooper & Vitousek 1998, Zaller & Arnone 1999, Stephan et al. 2000, Spehn et al. 2000), but also idiosyncratic and inconsistent results (Wardle & Nicholson, Mulder et al. 1999, Wardle et al. 1999, Gastine et al. 2003, Hedlund et al. 2003, Salamon et al. 2004).

Potential mechanisms involved in positive effects of plant diversity on decomposer performance include the following:

1. The increase in net primary production (NPP) which accompanies increasing plant species richness (Hector et al. 1999, Tilman et al. 2001, Spehn et al. 2005) beneficially affects the density and biomass of soil organisms.
2. The release of more diverse carbon compounds into the soil in the risosphere in more diverse plant communities. A greater diversity of plant exudates and rhizodeposits could result in a increased functional diversity of microbes and other decomposers (Li et al. 2004, Bais et al. 2004).
3. Increased litter diversity promotes greater decomposer diversity and hence increased litter processing. However, the effects of increasing litter diversity on decomposition rates have been found not only to be positive (Kaneko & Salamanca 1999, Bardgett & Shine 1999, Hector et al. 2000), but also negative or idiosyncratic (Hansen 2000, Wardle et al. 1997).

Decomposer effects on plants

Decomposer fauna affect the plants either directly via effects on seed viability, seedbank dynamics and seedling recruitment or indirectly by consuming detritus, releasing inorganic nutrients, and modifying the biomass, composition and activity of soil microbial communities. It has been shown that the composition of the soil food web affects plant nutrient uptake thereby significantly promoting plant growth (Bardgett & Chann 1999, Setälä & Huhta 1991, Scheu et al. 1999, Wurst et al. 2003) and plant competition (Kreutzer et al. 2004). There is evidence that some decomposer groups, particularly earthworms, contribute to the formation of soil seedbank and that the selective downward or upward transport of seeds, affects the composition of plant communities (Grant 1983, Pierce et al. 1994).

Despite the fact that decomposers appear to be rather inefficient in controlling microbial communities, microarthropods, such us mites or springtails and earthworms, may decrease the fungal to bacterial biomass ratio (Hanlon & Anderson 1979) and affect fungal competition through selective grazing (Parkinson et al. 1979, Tiunov & Scheu 2005).

1.3 Earthworms as decomposers

Aristotle was one of the first who pointed out the role of earthworms in turning over the soil and called them "The Intestines of the Earth". However, earthworms were considered in large pests until the publication of Charles Darwin's book "*The Formation of Vegetable Mould through the Action of Worms*", in 1881, where he convincingly documented in great detail the importance of earthworms in the breakdown of organic matter and the formation and maintenance of soil structure. Since then a wealth of studies has documented that earthworms play an essential role in soil formation, turnover of soil (Edwards & Bohlen 1996, Lavele et al. 1999), soil aeration and drainage (Edwards & Lofty 1978, Tisdall 1978, Carter et al. 1982), organic matter breakdown and incorporation into the soil (Satchell 1967, Edwards & Bohlen 1996), and nutrient mobilisation (Lee 1985, Edwards & Bohlen 1996). In recent years it has been stressed that the role of the earthworms does not stop belowground but that they also affect the aboveground subsystem, especially plant performance and plant community composition (Scheu et al. 1999, Wurst et al. 2003).

Scheu (2003) and Brown et al. (2004) identified seven main mechanisms by which earthworms can affect plants:

- *Direct interactions via:*

1. Root abrasion and ingestion of living plant parts.
2. Transposal of plant seeds.

- *Indirect interactios via:*

3. Changes in soil structure.
4. Changes in nutrient spatiotemporal availability.
5. Production of plant growth regulating substances.
6. Dispersal and changes in community structure and activity of beneficial microorganisms.
7. Changes in community structure of plant pests, parasites and pathogens in the rhizosphere.

Furthermore, it has been shown that the effect of earthworms on plants even cascades-up to consumers (herbivores) and affects aboveground multitrophic interactions (Scheu et al. 1999, Wurst & Johnes 2003).

1.4 Aims

As part of the soil fauna subproject in the "Jena Experiment" my work focused on the effects of soil macro-invertebrates (earthworms) and the interactions of earthworms with decomposer insects (collembolans) on the aboveground system in grassland communities of different diversity. As conceptual framework we approached the investigation simultaneously from above- and belowground evaluating how decomposer subsystem affects the aboveground subsystem, but also how the diversity above the ground impact decomposes.

In a greenhouse experiment we set up similar plant mixtures as in the field but at more controlled conditions and by using nitrogen tracers (^{15}N). We investigated on one hand how earthworms (and springtails) respond to variations in plant species and functional group diversity (Chapter 2) and on the other hand how the plant community responds to the presence of earthworms (and springtails) (Chapter 3).

Additionally, a second greenhouse experiment was set up investigating the role of earthworms as agents for seed displacement on the soil surface and burial of seeds into the soil (Chapter 4). Using the anecic earthworm species *Lumbricus terrestris* as a model species I studied whether seed displacement affects plant recruitment and ultimately the composition of plant communities.

Furthermore, earthworm densities were manipulated in the field to study the responses of plant species and functional group diversity to the increased and decreased density of earthworms (Chapter 5). In the field I also investigated how different plant mixtures affect the density, biomass and earthworm community composition and associated processes (litter decomposition, microbial biomass).

Chapter 2

The response of decomposers (earthworms, springtails and microorganisms) to variations in species and functional group diversity of plants

2.1 Abstract

The responses of three decomposer groups, (earthworms, springtails and microorganisms), to manipulations in plant species diversity (1, 2, 4, 8), plant functional group diversity (1, 2, 3, 4) and functional group identity (grasses, legumes, small herbs, tall herbs) were studied in a microcosm experiment. Separate and combined treatments with earthworms and springtails were set up. Two earthworm species representing major functional groups of earthworms in grasslands were investigated, the endogeic species *Aporrectodea caliginosa* (Savigny) and the anecic species *Lumbricus terrestris* L. For springtails three species were investigated, the hemiedaphic species *Heteromurus nitidus* (Leleup), *Folsomia candida* (Willem) and the euedaphic species *Protaphorura fimata* (Gisin). Plant species and functional group diversity beneficially affected *A. caliginosa* (increase in body weight and incorporation of ^{15}N from labelled litter) and *P. fimata* (density), presumably by changing the quality of belowground resources. In contrast, the biomass of *L. terrestris* decreased with plant species diversity but only in presence of legumes. For *H. nitidus* and *F. candida* the identity of plant functional groups was more important than plant species diversity per se. Also, the response of *F. candida* depended on earthworms. Microbial respiration was reduced by earthworms in more diverse plant communities, which correlated with root biomass. In contrast, microbial biomass was not affected by plant species diversity. The results suggest that belowground resource inputs from plant roots strongly modify decomposer performance and that the quality of the resources that enter the belowground subsystem is more important than their quantity. The responses of decomposers generally were not correlated with below- or aboveground plant productivity. In addition, the results document that the effect of plant community composition on the performance of decomposer species depend on the presence of other decomposers.

2.2 Introduction

Decomposers are crucial for transforming complex organic materials into inorganic forms without which dead organic material would accumulate irreversibly (Schlesinger 1997). The inextricable decomposer – producer co-dependency constitutes of the mineralization of organic matter by decomposers making nutrients available for producers to rebuilt complex organic matter. Despite the fact that decomposer microorganisms also immobilize inorganic nutrients which may result in competition with producers, the two compartments essentially complement each other (Harte & Kinzig 1993).

In attempts of linking below- and aboveground communities the feedback of plant communities to the decomposer food web is often neglected. The quantity and quality of resources produced by plant communities strongly influence the structure of soil food webs and their functioning (Wardle 2002, Scheu et al. 2003, De Deyn et al. 2004). Plant species effects manifest not only through the amount of litter returned to the soil (Wardle et al. 1995, Groffman el al. 1996) but also through the amount of soluble carbon compounds liberated via root exudates (Li et al. 2004, Bais et al. 2004), root and leaf chemical composition (Satchell 1967, Hendriksen et al. 1990, Tian et al. 1993) and the extent to which they deplete nutrients in the soil (Grime 1994, Fransen et al. 1999).

Earthworms and springtails are key decomposers affecting plant performance (Scheu et al. 1999, Wurst et al. 2003). Earthworms as soil macrofauna decomposers modify the physical structure of the soil (Lee & Foster 1991, Lavelle et al. 1997), alter soil microbial community composition and functioning (Brown 1995, Scheu 2002), increase nutrient cycling (Edwards & Bohlen 1996, Parmelee et al. 1989) and affect plant growth and vegetation development (Schmidt & Curry 1999, Zaller & Arnone 1999, Thompson et al. 1993, Scheu 2003). Springtails are important microbial grazers which affect the structure and functioning of the microbial community in the rhizosphere and plant nutrient availability (Rusek 1998, Gange 2000). Soil microorganisms compete with plants for nutrients (Kaye & Hart 1997, Hodge et al. 2000) and it has been documented that interactions between soil microorganisms and soil invertebrates significantly affect plant performance (Bonkowski & Scheu 2004). However, little is known how decomposers respond to differences in plant species and functional group diversity. The studies that investigated the effect of plant species diversity on the soil decomposer communities showed positive (Zaller & Arnone 1999, Stephan et al. 2000, Spehn

et al. 2000) or no consistent effects (Wardle et al. 1999, Gastine et al. 2003, Hedlund et al. 2003, Salamon et al. 2004).

In this study we established a microcosm experiment to investigate the response of different functional groups of decomposers to variations in plant species and plant functional group diversity. We expected changes in plant biomass, root exudation and microbial community composition caused by reductions in plant diversity to strongly affect the structure of the decomposer community and interactions between decomposer functional groups. Specifically we hypothesized that (1) an increase in plant species and functional group diversity beneficially affect decomposer performance, (2) decomposer performance varies with functional group and plant species identity, and (3) interactions between soil macrofauna decomposers and fungal grazing soil invertebrates depend on plant community composition. Using microcosms these hypothesis were evaluated under controlled conditions and well defined manipulations of the decomposer community. The use of ^{15}N labelled litter allowed to track nutrient fluxes from dead organic matter into plants and animals.

2.3 Materials and Methods

Experimental set-up

The experiment was set up in microcosms consisting of PVC tubes (inner diameter 10 cm, height 25 cm) which were sealed at the bottom with 40 µm mesh. The microcosms were filled with 1.4 kg of sieved (4 mm) soil (water content 13%). The soil (pH 8.1, carbon content 4.6%, C/N ratio 15.7) was taken from the northeast corner of the Jena Biodiversity Experiment field site (Thuringia, Germany; cf. Roscher et al. 2004). Prior to use the soil was defaunated by freezing at –22ºC for 14 days (Huhta et al. 1989). A layer of ^{15}N labeled roots of *Lolium perenne* (250 mg, 30 atom% ^{15}N; fragmented < 1 mm) was placed 2 cm below the soil surface. After placing in the microcosms the soil was irrigated by adding two 50-ml portions of deionized water every second day for 8 days to leach nutrients released as a result of the defaunation procedure. Subsequently, microcosms were kept moist for another 14 days by adding 50 ml deionized water every third day; weeds germinating during this period were removed.

Plant species were selected from a species pool representing Central European Arrhenatherion grasslands. A total of 43 plant species were used, grown from seeds in the defaunated soil and

transplanted into the microcosms when the plants had grown to a height of 2-6 cm. Eight plant individuals consisting of four functional groups (grasses, legumes, small herbs, tall herbs) were transplanted into each microcosm in different combinations following the design of the Jena Biodiversity Experiment (Roscher et al. 2004) (Appendix 3).

Table 2.1 Number of microcosms per combination of plant species number and number of plant functional groups.

Number of plant functional groups	Plant species number				
	1	2	4	8	
1	16	8	4	4	
2		8	4	4	
3			4	4	
4			4	4	
Number of microcosms	16	16	16	16	= 64

Plant functional groups were assessed using three classes of attributes: (1) above- and below-ground morphological traits (2) phenological traits and (3) the ability for N_2 fixation. The seventeen variables created from the selected species attributes were analysed by a multivariate cluster method (Ward's method, Euclidian distance (Kaufman & Rousseeuw 1990) in order to identify species functional groups (Roscher et al. 2004). Monocultures and species mixtures were established forming a plant species and functional group diversity gradient as given in Table 2.1. A total of 64 different plant species mixtures were set up.

Two grams of litter material consisting mainly of grass leaves was placed on top of the soil subsequently to the transplantation of plant seedlings. The litter material (2.53% N, C/N ratio 17.3) was collected near the site from which the soil had been taken, dried at 60ºC and cut into pieces about 3 cm in length.

One subadult *Aporrectodea caliginosa* (Savigny) and one juvenile of *Lumbricus terrestris* L. were added to half of the microcosms. *A. caliginosa* is an endogeic geophagous earthworm species, whereas *L. terrestris* is an anecic litter feeding species. Both species are among the dominant species at the Jena Biodiversity Experiment field site. Earthworms were weighed prior to placement in the microcosms (average fresh weight 863 and 927 mg for *A. caliginosa*

and *L. terrestris*, respectively). Twenty individuals of each of three Collembola species, *Heteromurus nitidus* (Leleup), *Folsomia candida* (Willem) and *Protaphorura fimata* (Gisin), were added to half of the microcosms creating four treatments in a two factorial design (Control, with Earthworms, with Collembola, with Earthworms and Collembola). The Collembola species were taken from laboratory cultures, where they were kept at constant temperature (17°C) and fed on bakery yeast. *Folsomia candida* and *Heteromurus nitidus* are hemiedaphic species dwelling in the litter layer and upper soil layers. *H. nitidus* is present at the Jena field site. *Protaphorura fimata* is an euedaphic species living in deeper soil layers. In total 256 microcosms were set up. During the experiment the microcosms were kept in a temperature controlled greenhouse at a day-night regime of 16-8 h and 20 ± 2ºC. During the experiment the water regime was increased from irrigating three times per week with 25 (week 1-2), 40 (week 3-5), 50 ml (week 5-7) deionized water to 50 (week 8-9) and 80 ml daily (week 9-11).

Sampling and analytical procedure

After 11 weeks earthworms were collected by hand sorting, washed, dried for 1 min on filter paper and weighed. Then, earthworms were killed by freezing, dried at 60ºC for three days and stored in a desiccator. The anterior end of *A. caliginosa* without gut content was used for analysing total nitrogen concentration and ^{15}N signatures which were determined by a coupled system consisting of an elemental analyzer (NA 1500, Carlo Erba, Milan) and a gas isotope mass spectrometer (MAT 251, Finngan; Reineking et al. 1993). For ^{15}N atmospheric N_2 served as primary standard and acetanilide (C_8H_9NO; Merk, Darmstadt) as internal calibration.

Collembola were sampled taking a soil core of a diameter of 5 cm from each of the microcosms to a depth of 5 cm. Collembola were extracted by heat (Macfadyen, 1961), separated into species and counted.

Microbial biomass was measured using the substrate-induced respiration (SIR) method (Anderson & Domsch 1978). The microbial respiratory response to addition of glucose was measured at hourly intervals in an electrolytic O_2 microcompensation apparatus for 24 hours at 22°C (Scheu 1992). Microbial biomass (C_{mic}; µg C g^{-1}soil) was measured after the addition of a sufficient amount of glucose as substrate in order to saturate the catabolic activity of microorganisms (4 mg glucose g^{-1} soil dry weight). The maximum initial respiratory response

(MIRR; µg O_2 g^{-1} soil dry weight h^{-1}) was calculated as the average of the lowest three readings within the first 11 h and microbial biomass was calculated as C_{mic} = 38 x MIRR (µg Cmic g^{-1} soil dry weight) (Anderson & Domsch 1978; Beck et al. 1997). Soil basal respiration (µl O_2 g^{-1} soil dry weight h^{-1}) was measured as mean of the O_2 consumption rates of unamended soil of hours 15 to 20 after start of the measurements.

We used analysis of variance (ANOVA) as part of the GLM procedure in SAS 8 (SAS Inst., Cary, Florida, USA) to test in a hierarchical order (type I sum of squares) the effects of earthworms (E), Collembola (C), plant species diversity (S), plant functional group diversity (FG) and presence/absence of legumes (L), grasses (G), small herbs (Sh) and tall herbs (Th) as treatment factors. The experimental design does not allow to fully separate the effects of S and FG which are partially confounded; the F-values given in text and tables for the effects of S (log-linear and deviation) and FG (linear and deviation), and their interactions with other factors refer to those where the respective factor (and interaction) was fitted first (Neter and Wasserman 1974, Schmid et al. 2002). No interaction term between S and FG was calculated. The effects of presence/absence of legumes (L), grasses (G), small herbs (Sh) and tall herbs (Th) and there interactions with earthworms and Collembola always were fitted after fitting S and FG. F-values of L x G interactions refer to those fitting the interaction before functional groups. In analyses of covariance (ANCOVA) plant shoot, root and total biomass were fitted as covariables to separate the effects driven by changes in plant primary production from diversity effects; covariables always were fitted before fitting S and FG (and their interactions with other factors). Microbial biomass and respiration were also analysed by ANCOVA using the soil water content as covariable to control for differences caused by soil moisture. Interactions between factors that were not significant were excluded from the model. Prior to ANOVA data were inspected for homogeneity of variance and log-transformed if required.

2.4 Results

Earthworms

Survival and body weight: In total, 92% of the 128 individuals of *A. caliginosa* added survived until the end of the experiment. On average, the biomass of *A. caliginosa* increased by 17%, however, the increase was significantly more pronounced in presence (+25%) than in absence of Collembola (+9%; Table 2.2). Furthermore, the body weight of *A. caliginosa*

increased with plant species and FG diversity but only in treatments without Collembola (Fig. 2.1a, b). Shoot, root and total plant biomass (fitted as covariables) did not significantly affect the biomass of *A. caliginosa* (P = 0.32, P = 0.83 and P = 0.34, respectively), suggesting that the plant species and functional group effects were not due to increased plant biomass.

The percentage of *L. terrestris* individuals collected at the end of the experiment was only around 60%; despite the 10 cm transparent fences used, some individuals managed to escape form the pots in the last weeks of the experiment when the plants were used to evade. On average the biomass of the surviving individuals had increased by 34%.

In contrast to *A. caliginosa* the presence of Collembola did not affect the body weight of *L. terrestris*, but it decreased with plant species diversity in treatments with legumes, whereas in treatments without legumes it was at a maximum at the maximum plant species diversity (S x L interaction $F_{3,42} = 4.06$, P = 0.0128; Fig. 2.1c).

Tissue nitrogen: Tissue nitrogen concentration was only analysed for *A. caliginosa*. It was affected by plant functional group diversity but only in the treatment with Collembola being at a minimum at the three functional group diversity level (significant C x FG interaction; Table 2.2). The effect of plant functional group diversity likely was caused by legumes (significant C x L interaction; Table 2.2); without legumes Collembola decreased the concentration of nitrogen in earthworm tissue from 13.1% to 12.8% suggesting that Collembola and earthworms competed for nitrogen resources but only if there were no legumes (Fig. 2.2a).

^{15}N incorporation: Incorporation of ^{15}N from the litter was only analysed for *A. caliginosa*. Similar to tissue nitrogen concentration the ^{15}N atom% in *A. caliginosa* depended on Collembola and plant functional group diversity, with legumes contributing most to this effect (Table 2.2). In presence of legumes and without Collembola earthworm tissue ^{15}N atom% slightly increased; in contrast, in presence of Collembola it decreased (Fig. 1.2b). Again, this suggests that presence of legumes provided additional nitrogen that diminished the competition for nitrogen between *A. caliginosa* and Collembola that occurred in the absence of legumes. As a result the total earthworm N tissue content did not decrease but the ^{15}N atom% declined, presumably in part through assimilation of nitrogen with low ^{15}N signature typical for legume fixed nitrogen.

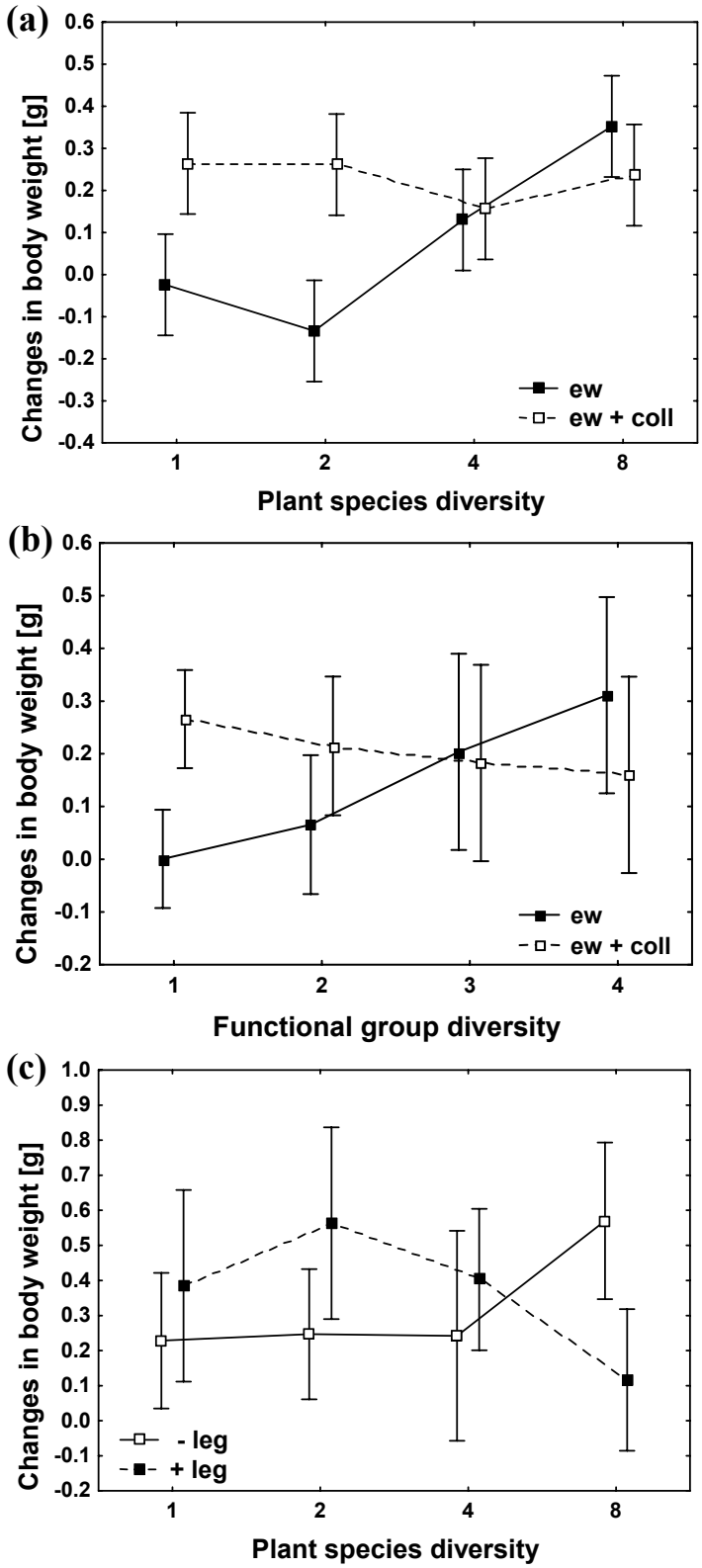

Figure 2.1 (a) Body weight of *Aporrectodea caliginosa* as affected by plant species diversity and Collembola, (b) plant functional group diversity and Collembola, and (c) body weight of *Lumbricus terrestris* as affected by plant species diversity and presence of legumes. Error bars represent ± SE.

Table 2.2 ANOVA table of F-values on the effect of Collembola (C), number of plant species (S), number of plant functional groups (FG) and presence of legumes (L), grasses (G), small herbs (Sh) and tall herbs (Th) on changes in body weight, tissue nitrogen concentrations and ^{15}N atom% in *Aporrectodea caliginosa.*

Variables analysed / **Treatment factors**	**Changes in body weight**	**Tissue N (%)**	**^{15}N atom%**
C	**$F_{1/97} = 15.75$*****	$F_{1/82} = 0.39$	**$F_{1/82} = 4.54$***
FG	$F_{3/97} = 1.39$	$F_{3/82} = 2.21^{+}$	**$F_{3/82} = 3.61$***
FG linear	$F_{1/124} = 2.63$	$F_{1/109} = 0.01$	$F_{1/109} = 3.34^{+}$
FG deviation	$F_{2/124} = 0.12$	$F_{2/109} = 3.00^{+}$	$F_{2/109} = 3.00^{+}$
S	**$F_{3/97} = 5.78$****	$F_{3/82} = 0.81$	$F_{3/82} = 2.62^{+}$
S log-linear	**$F_{1/124} = 8.25$****	$F_{1/109} = 0.21$	$F_{1/109} = 3.64^{+}$
S deviation	$F_{2/124} = 2.30$	$F_{2/109} = 0.99$	$F_{2/109} = 1.49$
L	$F_{1/97} = 0.56$	$F_{1/82} = 3.65^{+}$	**$F_{1/82} = 5.33$***
G	$F_{1/97} = 0.12$	$F_{1/82} = 0.31$	$F_{1/82} = 1.59$
Sh	$F_{1/97} = 0.16$	$F_{1/82} = 1.29$	$F_{1/82} = 0.04$
Th	$F_{1/97} = 0.49$	$F_{1/82} = 0.06$	$F_{1/82} = 1.87$
C x FG	**$F_{3/97} = 4.31$****	**$F_{3/82} = 3.09$***	$F_{1/82} = 0.74$
C x S	**$F_{3/97} = 8.24$*****	$F_{3/82} = 1.04$	$F_{1/82} = 1.00$
C x L	$F_{3/97} = 0.78$	**$F_{1/82} = 7.03$****	**$F_{3/82} = 8.44$****
C x G	$F_{3/97} = 0.14$	$F_{3/82} = 3.12^{+}$	$F_{3/82} = 3.28^{+}$
C x Sh	$F_{3/97} = 1.43$	$F_{3/82} = 0.18$	$F_{3/82} = 2.74$
C x Th	$F_{3/97} = 0.46$	$F_{3/82} = 2.10$	$F_{3/82} = 0.25$
S x L	**$F_{3/97} = 2.93$***	$F_{3/82} = 0.64$	$F_{3/82} = 2.65^{+}$
S x G	**$F_{3/97} = 3.17$***	$F_{3/82} = 1.40$	$F_{3/82} = 1.13$
S x Sh	$F_{3/97} = 0.64$	$F_{3/82} = 0.61$	$F_{3/82} = 1.10$
S x Th	**$F_{2/97} = 2.90$***	$F_{2/82} = 1.72$	$F_{2/82} = 0.65$

*****, P < 0.001; **, P < 0.01; *, P < 0.05; $^{+}$, P < 0.10**

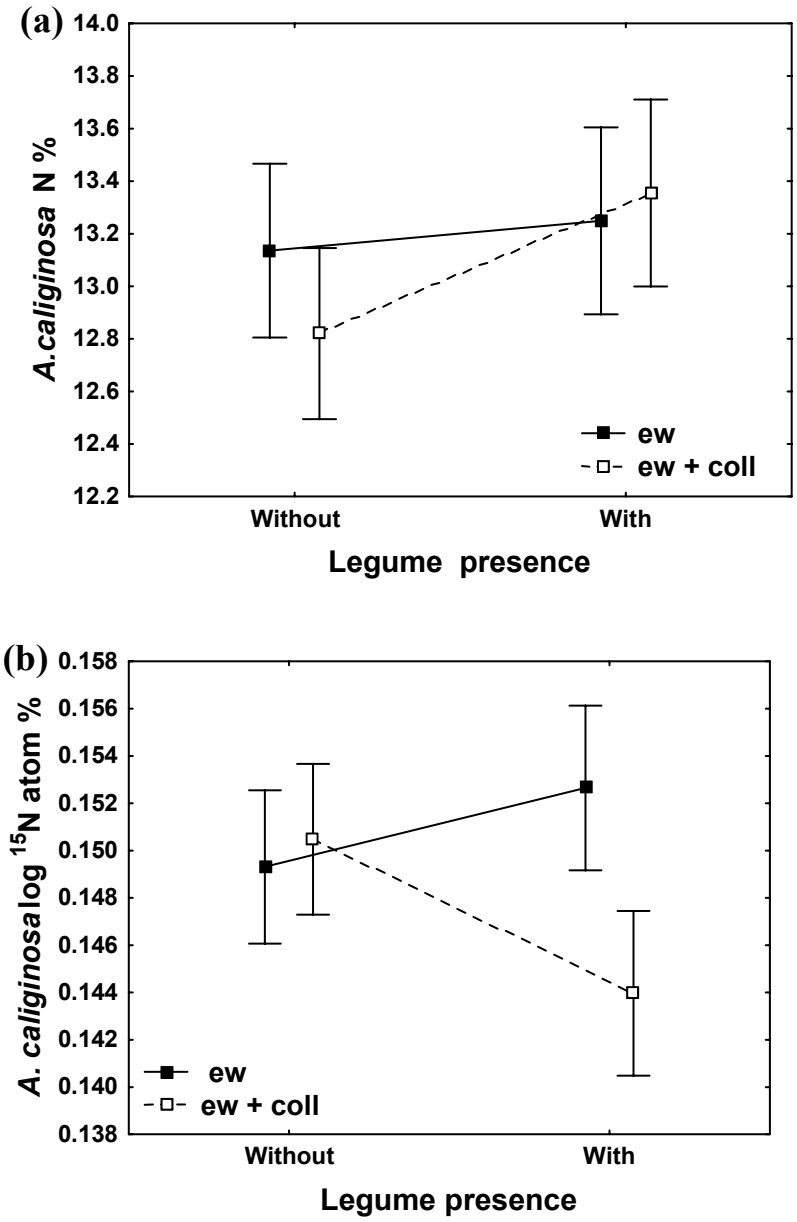

Figure 2.2 (a) Tissue nitrogen concentration and (b) ^{15}N atom% of *Aporrectodea caliginosa* as affected by Collembola and presence of legumes. Error bars represent ± SE.

Microorganisms

Microbial basal respiration but not microbial biomass was significantly affected by soil water content as indicated by ANCOVA (Table 2.3). Presence of earthworms reduced microbial basal respiration by 17%, whereas Collembola did not affect microbial respiration (Fig. 2.3a). Microbial basal respiration but not microbial biomass was significantly affected by plant species and functional group diversity, decreasing log-linearly and linearly, respectively, with

the increase in species and functional group diversity (Fig. 2.3b, c). Basal respiration was at a maximum in the two species treatment and at a minimum in the eight species treatment with the one and four species treatment being intermediate. In presence of tall herbs basal respiration was increased by ca. 5%.

Microbial biomass was only affected by earthworms; in presence of earthworms it decreased on average by approximatively 4%. Including root biomass, total plant biomass and the biomass ratio between plant functional groups per pot as covariables suggest that root biomass contributed to the plant diversity effect on basal respiration (drop of P-values to 0.0644), but these parameters did not contribute to the reduction in microbial biomass in presence of earthworms nor to the effect of tall herbs on soil respiration.

Table 2.3 ANCOVA table of F-values on the effect of Earthworms (E), Collembola (C), number of plant species (S), number of plant functional groups (FG) and presence of legumes (L), grasses (G), small herbs (Sh) and tall herbs (Th) on microbial basal respiration and microbial biomass; soil water content was used as covariable.

Variables analysed **Treatment factors**		**Basal respiration**	**Microbial biomass**
Water		**$F_{1/237}$ = 52.32*** **	$F_{1/237}$ = 0.28
E		**$F_{1/237}$ = 43.44*** **	**$F_{1/237}$ = 7.15** **
C		$F_{1/237}$ = 0.01	$F_{1/237}$ = 0.87
E x C		$F_{1/237}$ = 1.76	$F_{1/237}$ = 0.25
FG		$F_{3/237}$ = 1.34	$F_{3/237}$ = 1.29
	FG linear	**$F_{1/247}$ = 9.51** **	$F_{1/247}$ = 1.79
	FG deviation	$F_{1/247}$ = 0.68	$F_{1/247}$ = 1.03
S		**$F_{3/237}$ = 3.13***	$F_{3/237}$ = 1.03
	S log linear	**$F_{1/247}$ = 5.85***	$F_{1/247}$ = 0.71
	S deviation	$F_{1/247}$ = 2.47	$F_{1/247}$ = 1.15
G		$F_{1/237}$ = 2,05	$F_{1/237}$ = 1.53
Sh		$F_{1/237}$ = 0.52	$F_{1/237}$ = 3.41
Th		**$F_{1/237}$ = 6.95** **	$F_{1/237}$ = 0.03
L		$F_{1/237}$ = 0.81	$F_{1/237}$ = 1.70

*****, P < 0.001; **, P < 0.01; *, P < 0.05;**

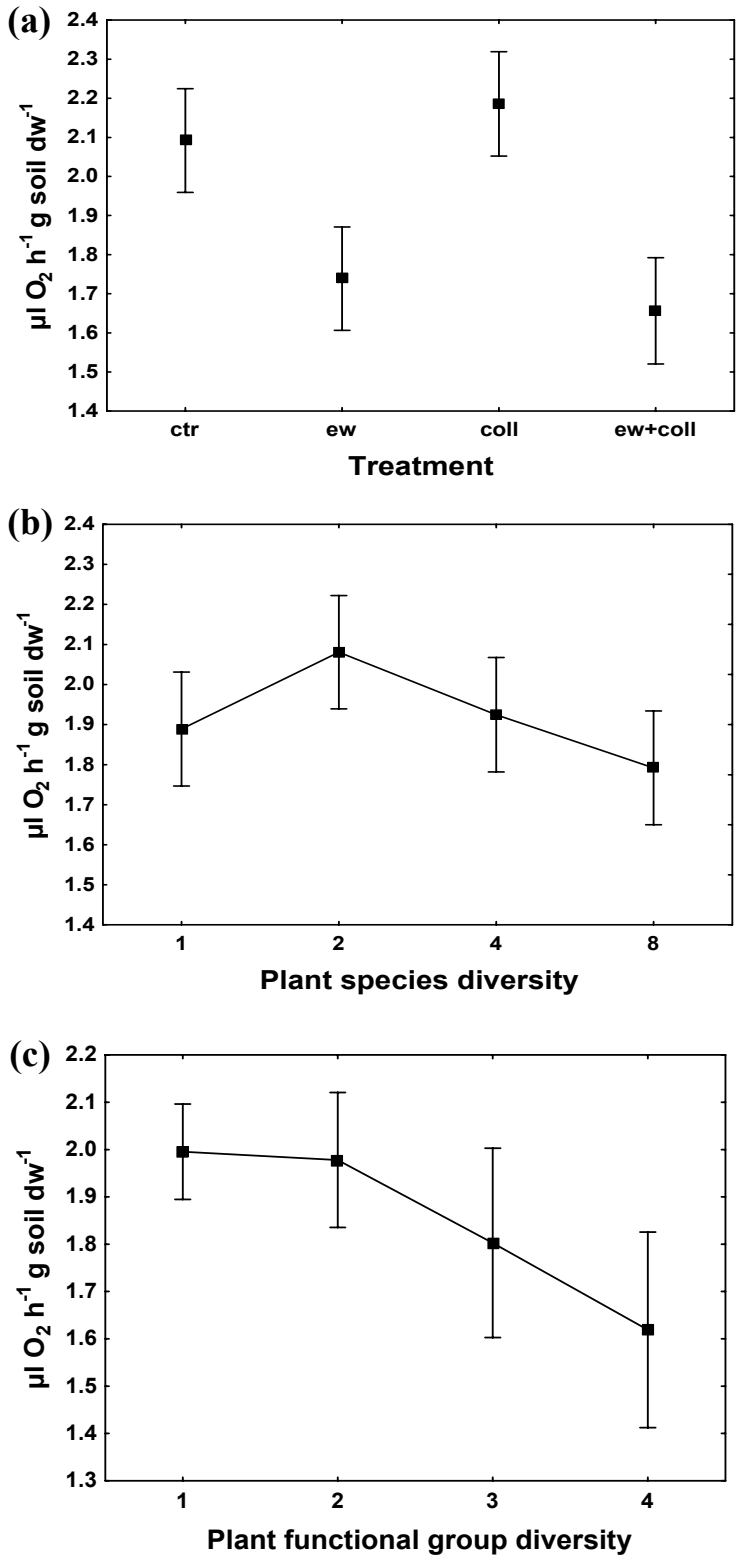

Figure 2.3 Microbial basal respiration as affected by (a) decomposers, (b) plant species diversity and (c) plant functional group diversity. Adjusted means and standard deviation calculated using water as covariable (average water content 30.8%). Error bars represent ± SE.

2.5 Discussion

Earthworms

Earthworms are considered to be strongly influenced by the amount of plant residues entering the soil (Edwards and Bohlen 1996). Since most of the biomass produced by plants ultimately enters the detrital system earthworms should benefit from increased primary production. Since primary production increases with plant species richness in grassland communities (Hector et al. 1999, Tilman et al. 2001) plant species diversity likely also impacts earthworms and other decomposers. In fact, in field experiments Zaller & Arnone (1999) and Spehn et al. (2000) found the biomass of earthworms to increase with increasing plant species richness. In both studies this was explained by increased input of resources but also by changes in resource quality. In field experiments it is difficult to disentangle effects of resource quality from those of resource quantity (Scheu & Schaefer 1998, Maraun et al. 2001). Furthermore, it is difficult to differentiate between effects caused by litter materials from above the ground from those caused by root derived resources. Results of the present study suggest that the increase in earthworm biomass with increasing plant species diversity is largely caused by root derived resources since the same aboveground litter resources were added to each of the treatments. However, results of ANCOVAs suggest that the increase in body weight of *A. caliginosa* with increase in plant species richness was not related to root or total plant biomass. Presumably, the increase in earthworm body weight with increasing plant diversity was caused by changes in the quality rather than the quantity of rhizodeposits. Possibly, rhizodeposits are more diverse in more diverse plant communities. In contrast to the study of Spehn et al. (2000), the increase in body weight of *A. caliginosa* was not related to the presence of legumes which provide shoot and root litter resources rich in nitrogen. This supports the conclusion of Tiunov and Scheu (2004) that endogeic earthworms are primarily limited by carbon rather than nitrogen. Interestingly, the increase in biomass of *A. caliginosa* in more diverse plant communities was associated with an increase in the exploitation of the ^{15}N labelled litter material added to the microcosms. This indicates that earthworms in the more diverse plant communities were more active and therefore more efficiently exploited organic resources in soil.

A striking result of the present study was that at low plant diversity the increase in body weight of *A. caliginosa* was more pronounced in presence of Collembola. This suggests that

Collembola facilitated the resource acquisition by earthworms. However, as indicated by tissue ^{15}N concentrations this was not the case for the litter resources added to the microcosms. Also, reduced tissue nitrogen concentration of *A. caliginosa* in presence of Collembola (treatments without legumes) suggests that Collembola and earthworms competed for nitrogen resources which is consistent with earlier studies (Scheu et al. 1999). Overall, the results indicate that the relationship between earthworms and Collembola is complex; depending on the diversity of the plant community and the element considerd (C or N) Collembola may facilitate or inhibit earthworm resource acquisition.

Compared to the endogeic species *A. caliginosa* which predominantly feeds on organic resources in the soil the anecic species *L. terresris* was less responsive to the experimental treatments. Anecic species strongly rely on litter input form above the ground (Edwards & Bohlen 1996). Since each of the treatments was set up with the same litter material the lower responsiveness of *L. terrestris* was expected. However, changes in body weight of *L. terresris* also were affected by plant species diversity but this varied significantly with the presence of legumes. Without legumes earthworm body weight only increased in the highest plant diversity treatment whereas in presence of legumes it was at a maximum in the two species treatment and then declined at higher plant species diversity. The increase in biomass of *L. terrestris* suggests that anecic earthworm species also rely at least in part on belowground resources. The increase in biomass at high plant species diversity (in absence of legumes) supports our conclusion that earthworms may benefit from more diverse rhizodeposits.

Microorganisms

Microorganisms in soil primarily are limited by the amount of carbon entering the detrital system and hence by plant biomass production (Zak et al. 1994, Spehn et al. 2000). However, in our experiment microbial biomass was not related to total plant biomass production but correlated weakly with root biomass. This suggests that microbial biomass in soil (as measured by substrate-induced respiration) is resistant to plant species composition (and associated belowground resource input) and to the feeding activity of Collembola. In agreement with these findings it has been documented that even massive changes in liquid carbon input (glucose) hardly affect microbial biomass in soil (Joergensen and Scheu 1999, Maraun et al. 2001). The failure of Collembola to control the biomass of microorganisms in soil suggests that most of the microorganisms are inaccessible to microarthropod grazers

(Schlatte et al. 1998, Kandeler et al. 1998). In contrast to Collembola, microbial biomass was reduced in presence of earthworms suggesting that either earthworms digested microorganisms or effectively competed with microorganisms for resources. There is increasing evidence that the latter but not the former is in fact the case (Wolter & Scheu 1999, Schönholzer et al. 1999, Scheu & Schaefer 1998, Tiunov & Scheu 2004). Overall, the low responsiveness of microbial biomass to the experimental treatments suggests that the effects of plant species and functional group diversity on the performance of earthworms and Collembola directly resulted from changes in plant resources rather than indirectly from plant-mediated changes in microbial biomass. The low responsiveness of microbial biomass suggests that microorganisms in soil are rather resistant to changes in belowground resource supply.

In comparison to microbial biomass, the respiratory activity of microorganisms responded more sensitively to the experimental manipulations. Similar to microbial biomass microbial respiration was also reduced by earthworms supporting our conclusion that earthworms effectively competed with microorganisms for resources in soil. Furthermore, microbial respiration varied with plant species diversity and this likely was in part due to differences in root biomass. However, the significant effect of tall herbs on microbial respiration and the lack of correlation with root biomass suggest that not only belowground productivity but also the quality of rhizodeposits affects microbial activity in the rhizosphere.

The hypothesis that decomposers are beneficially affected by an increase in plant species and functional group diversity appears to be oversimplistic. Rather, the response of decomposers to variations in plant diversity varies with decomposer species with endogeic and euedaphic species, such as *A. caliginosa* and *P. fimata* being more sensitive than anecic (*L. terrestris*) or hemiedaphic species (*F. candida* and *H. nitidus*). Consistent with our expectations the identity of the plant functional groups strongly affected growth and reproduction of decomposers, Grasses beneficially affected Collembola densities whereas legumes detrimentally affected Collembola densities but beneficially affected total N concentration in *A. caliginosa* tissue. Also, consistent with our expectations soil macrofauna and mesofauna species affected each other and these interactions were modified by plant species diversity, plant functional group diversity and presence of legumes. Both changes in the amount and quality of belowground resources presumably were responsible for these modifications. Future experiments need to further incorporate variations in the input of aboveground litter resources with plant species

and functional group diversity to fully capture the complexity of the dependency of decomposers on plant community composition.

Chapter 3

The role of decomposer animals (Lumbricidae, Collembola) for plant performance in model grassland systems of different diversity

3.1 Abstract

Decomposer invertebrates influence soil structure and nutrient mineralisation as well as the activity and composition of the microbial community in soil and therefore, likely affect plant performance and plant competition. Model grassland communities were established in the greenhouse to study the interrelationship between two different functional groups of decomposer invertebrates, Lumbricidae and Collembola, and their effect on plant performance and plant nitrogen uptake. Common plant species of Central European Arrhenatherion grasslands were transplanted into microcosms with numbers of plant species varying from 1 to 8 and plant functional groups varying from 1 to 4. Separate and combined treatments with earthworms and collembolans were set up. Microcosms contained ^{15}N labelled litter to track N fluxes into plant shoots. Increasing plant functional group diversity increased total plant biomass and shoot biomass, each being at a maximum in the eight species mixtures. Increasing plant species diversity increased shoot biomass but the effect varied with plant functional group identity. Presence of legumes increased total plant biomass and shoot biomass, whereas presence of grasses and tall herbs decreased total plant and plant shoot biomass. Presence of small herbs increased shoot biomass only. Presence of decomposers strongly increased total plant biomass and shoot biomass. Root biomass decreased in presence of collembolans and even stronger in presence of earthworms. However, it increased when both animal groups were present. Also, presence of decomposers increased total N concentrations and ^{15}N enrichment of grasses, legumes and small herbs. The latter was at a maximum in the combined treatment with earthworms and collembolans. The impact of earthworms and collembolans on plant performance strongly varied with plant functional group identity and tended to vary with plant species diversity. Both decomposer groups appeared to generally promote aboveground plant productivity by their effects on litter decomposition and nutrient mineralisation leading to an increased plant nutrient acquisition.

The non-uniform effects of earthworms and collembolans demonstrate that functional diversity of soil decomposer is an important structuring force for aboveground plant community composition.

3.2 Introduction

In terrestrial ecosystems soil decomposer animals are essential for nutrient mineralisation (Bradford et al. 2002) and alter the availability of nutrients to plants (Wardle 1999). While microorganisms dominate mineralisation processes, their activity, community composition and spatial distribution is strongly modified by soil invertebrates (Scheu & Setälä 2002, Bonkowski & Scheu 2004). For example, the feeding activity of earthworms and collembolans on bacteria and fungi indirectly affect the availability of nutrients in soils (Wardle 1999). It is well documented that the enhanced nutrient turnover in soil in presence of decomposer animals leads to a higher plant nutrient acquisition and therefore stimulates plant growth (Scheu et al. 1999, Schmidt & Curry 1999, Kreuzer et al. 2004).

Previous studies suggested that some soil animal species are functionally redundant and do have no detectable influence on ecosystem functions such as N mineralisation and plant growth (e.g. Cragg & Bardgett 2001, Liiri et al. 2002). In contrast, Cole et al. (2004) documented that increasing species richness of soil microarthropods increased shoot biomass and total N in soil leachates. However, for maintaining ecosystem processes the functional characteristics of species likely are more important than the number of species per se (Laakso & Setälä 1999, Cragg & Bardgett 2001, Cole et al. 2004) due presumably to differential effects of animal groups on, nutrient fluxes, and on structure and dynamics of the soil microbial community by different (Bardgett & Chan 1999).

In terms of biomass earthworms are among the most important detritivore animals in terrestrial ecosystems (Edwards & Bohlen 1995). Especially in grasslands they are known to play a key role in nutrient cycling and physical soil improvement (Spehn et al. 2000), and therefore increase plant growth. Earthworms influence plant performance either direct, e.g. via root feeding and translocation of seeds, or indirect via altering microbial activity and plant nutrient availability. Direct effects probably are rare and usually less important than indirect effects. However, the mechanisms responsible for earthworm-mediated changes in plant performance in most cases are unknown. Scheu et al. (1999) and Kreuzer et al. (2004) showed

that the effect of earthworms varies with plant species and is more pronounced in grasses than in legumes suggesting that earthworm effects vary with plant functional groups.

Collembolans are among the most abundant soil arthropods feeding on a range of resources (Hopkin 1997). By consuming dead plant material and fungal hyphae, collembolans might play an important role in enhancing decomposition processes since hyphal grazing stimulates growth and respiration of fungi (Gange 2000). Thus, collembolans predominantly influence plant performance and plant competition indirectly by altering microbial activity and microbial community structure, and therefore the competition for nutrients between microbes and plants.

Stimulation of plant performance by decomposer invertebrates is well known, but only few investigations documented that different functional groups of decomposer invertebrates affect plant competition and therefore plant community structure (Scheu & Setälä 2002, Wardle 2002, Kreuzer et al. 2004). Studies examining the role of decomposers for plant growth focused on single target plant species or the competition between two plant species belonging to different plant functional groups. This is the first experiment investigating effects of different functional groups of decomposer invertebrates on plant communities of different species and functional group diversity. In a microcosm experiment in a greenhouse we investigated under controlled conditions (i) the effect of two decomposer animal groups, earthworms and collembolans, on plant growth and plant nitrogen uptake of communities differing in plant species and functional group diversity, (ii) variations of the effect with plant functional group identity, and (iii) mechanisms responsible for these changes by using ^{15}N labelled litter material to track nutrient fluxes from litter into plant tissue. As a basis, the impact of plant species and plant functional group diversity and identity on plant performance was analysed.

3.3 Materials and Methods

Microcosms

Experimental containers consisted of Perspex tubes (inner diameter 10 cm, height 25 cm), sealed with a 45 µm mesh at the bottom and a plastic barrier (10 cm height) at the upper end, to avoid escape of animals. A total of 256 microcosms were filled with soil (1.4 kg fresh weight) from *The Jena Biodiversity Experiment* field site (Jena, Thuringia, Germany; Roscher

et al. 2004), including a layer of 250 mg of ^{15}N labelled roots (30 atom % ^{15}N; fragmented < 1 mm) of *Lolium perenne* L. placed 2 cm below the soil surface. Prior to use the soil (Eutric Fluvisol; FAO Unesco (1997); sand content 15%, water content 13%, pH 8.1, nitrogen content 0.3%, carbon content 4.6%, C-to-N ratio 15.7) was sieved (4 mm) and defaunated by freezing for two weeks at -22°C (Huhta et al. 1989). After placement into the microcosms the soil was irrigated by adding two 50 ml-portions of deionized water every second day for eight days to leach nutrients released as a result of the defaunation procedure. Subsequently, microcosms were kept moist for another 14 days adding one portion of 50 ml deionized water each third day; germinating weeds were removed.

Eight pre-germinated plant individuals (height 2-6 cm) of a total of 43 common species of Central European Arrhenatherion grasslands, consisting of four functional groups (grasses (G), legumes (L), small herbs (Sh), tall herbs (Th)) were transplanted into each microcosm in different combinations following the design of *The Jena Experiment* with plant species diversity varying from 1 to 8 and plant functional group diversity varying from 1 to 4 (Roscher et al. 2004; Table 2.1).

Altogether 64 plant species combinations were established (Appendix 3): 16 monocultures (divided in functional groups: 4 x G, 4 x Sh, 4 x Th, 4 x L), 16 pots each with 2 species (2 x G, 2 x Sh, 2 x Th, 2 x L, 2 x GSh, 2 x ThL, 2 x GTh, 2 x ShL), 16 pots each with 4 species (1 x G, 1 x Sh, 1 x Th, 1 x L, 1 x GSh, 1 x ThL, 1 x GTh 1 x ShL, 1 x GShTh, 1 x GThL, 1 x GShL, 1 x ShThL, 4 x GShThL) and 16 pots each with 8 species (1 x G, 1 x Sh, 1 x Th, 1 x L, 1 x GSh, 1 x ThL, 1 x GTh 1 x ShL, 1 x GShTh, 1 x GThL, 1 x GShL, 1 x ShThL, 4 x GShThL) (Appendix 3). Subsequently, additional 2 g of non-labelled litter material (2.53% N, C/N ratio 17.3) were placed on the soil surface. The litter consisted predominantlly of grasses and had been collected from the Jena field site, dried at 60°C for three days and cut into pieces of 3 cm length.

Earthworms (one individual of each of *Lumbricus terrestris* (L.) and *Aporrectodea caliginosa* (Savigny)) and collembolans (20 individuals of each of *Protaphorura fimata* (Gisin), *Heteromurus nitidus* (Leleup) and *Folsomia candida* (Willem)) were added to half of the microcosms to establish four treatments in a two factorial design: no animals (control), earthworms only, collembolans only, earthworms and collembolans combined. *A. caliginosa* is an endogeic geophagous earthworm species, whereas *L. terrestris* is an anecic litter feeding species. Both species are among the dominant species at the field site of "*The Jena*

Biodiversity Experiment". All three collembolan species were taken from laboratory cultures, where they were kept at constant temperature (17°C) and fed on bakers yeast. *Folsomia candida* and *Heteromurus nitidus* are hemiedaphic species dwelling in the litter layer and upper soil layers; *Protaphorura fimata* is a euedaphic species living in deeper soil layers.
Microcosms were incubated for eleven weeks at a day-night regime of 16-8 h and 20 ± 2°C and the water regime was successively increased from irrigating three times a week with 25 (week 1-2), 40 (week 3-5), 50 ml (week 5-7) deionized water to 50 (week 8-9) and 80 ml daily (week 9-11). Microcosms were randomised every 3 weeks.

Sampling

Flowers were counted once a week starting in week 5 of the experiment, and at the end of the experiment. Seeds of *Plantago lanceolata*, *P. media* and *Medicago lupulina*, the only species which abundantly produced seeds, were harvested and weighed. After eleven weeks plants were harvested. Shoots were cut at the soil surface, separated into species and dried at 60°C for three days. Roots were washed out of the soil using a 1 mm mesh and dried at 60°C for three days. For tracing the pathway of nitrogen in the labelled litter we chose one plant of each of three plant functional groups which were present in most of the 64 different plant combinations, i.e. *Onobrychis viciifolia* (legume), *Festuca rubra* (grass) and *Plantago lanceolata* (small herb).

Total nitrogen concentrations and ^{15}N signatures of *Onobrychis viciifolia*, *Festuca rubra* and *Plantago lanceolata* were determined by a coupled system consisting of an elemental analyser (NA 1500, Carlo Erba, Milan) and a gas isotope mass spectrometer (MAT 251, Finngan; Reineking et al. 1993). For ^{15}N atmospheric N_2 served as the primary standard and acetanilide (C_8H_9NO; Merk, Darmstadt) was used for internal calibration.

Earthworms were collected by hand sorting. Collembolans were sampled taking soil cores (diameter 5 cm) from each of the microcosms and extracted by heat. Data on decomposer animals are presented in Chapter 2.

Statistical analyses

Data on shoot and root biomass were summed up per pot. Data on flowers and seeds were included in aboveground biomass data, but also analysed separately. Due to low numbers of

flowers and seeds this was mainly done by calculating correlations using Statistica 6.0 (StatSoft Inc., Hamburg, Germany).

In order to seperate legume (which were very dominant) and non-legume responses we analysed total shoot biomass (including legumes) and shoot biomass without legumes. Total shoot biomass was used to calculate total biomass (shoots and roots) per pot and shoot-to-root ratio per pot. Also, for total shoot biomass the mean weight of individual shoots of each of the plant functional groups was calculated per pot, and total N concentration and ^{15}N enrichment of the selected plant species were measured.

The effect of earthworms (EW), collembolans (COL), number of plant species (S; log_2-linear and deviation) and number of plant functional groups (FG; linear and deviation) on each variable was determined using type I analysis of variance in a general linear model (GLM). Differences between means were inspected using Tukey´s honestly significant difference test (HSD, $\alpha = 0.05$; Sokal & Rohlf 1995). Data were tested for normal distribution and homogeneity of variance (Levene-Test) and $\log_{10}(x + 0.1)$-transformed if necessary. The experimental design does not allow to fully separate the effects of S and FG which are partially confounded. Therefore, no interaction between S and FG was calculated. The effects of presence and absence of L, G, Sh and Th always were fitted after fitting S and FG. The F-values given in text and tables for the effects of S, FG, L, G, Sh and Th refer to those where the respective factor, and interaction, was fitted first (Schmid et al. 2002). Statistical analyses were performed using Statistical Analysis System 8.2 (SAS Institute, Cary, N.C., USA).

3.4 Results

Seeds and flowers

Of the 43 species we used in the present study, only three legumes (*Lotus corniculatus*, *Trifolium hybridum*, *Trifolium pratense*), two grasses (*Trisetum flavescens*, *Poa trivialis*) and two small herbs (*Leontodon autumnalis*, *Leontodon hispidus*) produced flowers during the experiment. Total number of flowers decreased with increasing plant species diversity from 69.6 ± 22.5 to 10.4 ± 2.5 ($F_{2,53} = 24.11$, $P < 0.0001$). None of the plants in the single species treatments flowered. Also, total number of flowers decreased with increasing plant functional group diversity from 41.6 ± 7.1 to 10.6 ± 7.3 ($F_{3,35} = 7.89$, $P = 0.0002$). Presence of collembolans significantly reduced the number of flowers of *Trifolium pratense* from 22.6 ±

5.6 to 17.2 ± 5.5 ($F_{1,5}$ = 12.24, P = 0.017), whereas presence of only collembolans (19.2 ± 4.6) and even stronger presence of only earthworms (28 ± 5.1) increased the number of flowers of *Trifolium hybridum* compared to the control treatment (9 ± 4.6); the effect was less pronounced in the combined treatment with earthworms and collembolans (16.2 ± 4.6; EW x COL interaction; $F_{1,11}$ = 8.79, P = 0.013).

Due to the absence of pollinators in the greenhouse, only *Plantago lanceolata*, *Plantago media* and *Medicago lupulina* produced seeds and seed production was limited to plant mixtures with four (7.9 ± 3.4 seeds; 0.2 ± 0.07 g) and eight (23.3 ± 3.4 seeds; 0.5 ± 0.07 g) plant species and two (14.2 ± 3.3 seeds; 0.3 ± 0.07 g) and four (34.3 ± 4.7 seeds; 0.9 ± 0.1 g) plant functional groups, respectively. Still, increasing plant species and plant functional group diversity but not presence of earthworms and collembolans significantly increased total number (S: $F_{3,244}$ = 11.77, P < 0.0001; FG: $F_{3,244}$ = 29.79, P < 0.0001) and total weight of seeds (S: $F_{3,244}$ = 16.38, P < 0.0001; FG: $F_{3,244}$ = 27.02, P < 0.0001). However, the number of seeds of *Plantago media* decreased with the density of *Protaphorura fimata* in the microcosms at the end of the experiment (r^2 = 0.875, P = 0.02).

Shoot biomass

Shoot biomass increased with increasing plant species diversity, ranging from 5.9 ± 0.3 g to 6.9 ± 0.3 g, and, even more pronounced, with plant functional group diversity, ranging from 5.8 ± 0.2 g to 7.7 ± 0.4 g (Fig. 3.1a, b, Table 3.2). Also, each of the plant functional groups strongly affected shoot biomass, but the effects varied with increasing plant species and plant functional group diversity. Overall, grasses (from 6.7 ± 0.2 g to 5.7 ± 0.2 g) and tall herbs (from 6.5 ± 0.2 g to 6.0 ± 0.2 g) decreased shoot biomass, whereas legumes (from 4.9 ± 0.1 g to 7.8 ± 0.2 g) and small herbs (from 5.7 ± 0.2 g to 7.0 ± 0.2 g) increased it. The generally negative effect of grasses was strongest in the two species mixtures (Fig. 3.1c, Table 3.2), whereas tall herbs decreased shoot biomass in the one, two and four species mixtures, but slightly increased it in the eight species mixtures (Table 3.2). Legumes generally increased shoot biomass, but the effect was more pronounced at higher species diversity levels (Fig. 3.1d, Table 3.2), whereas small herbs had the strongest positive effect on shoot biomass in the one species treatments (Table 3.2).

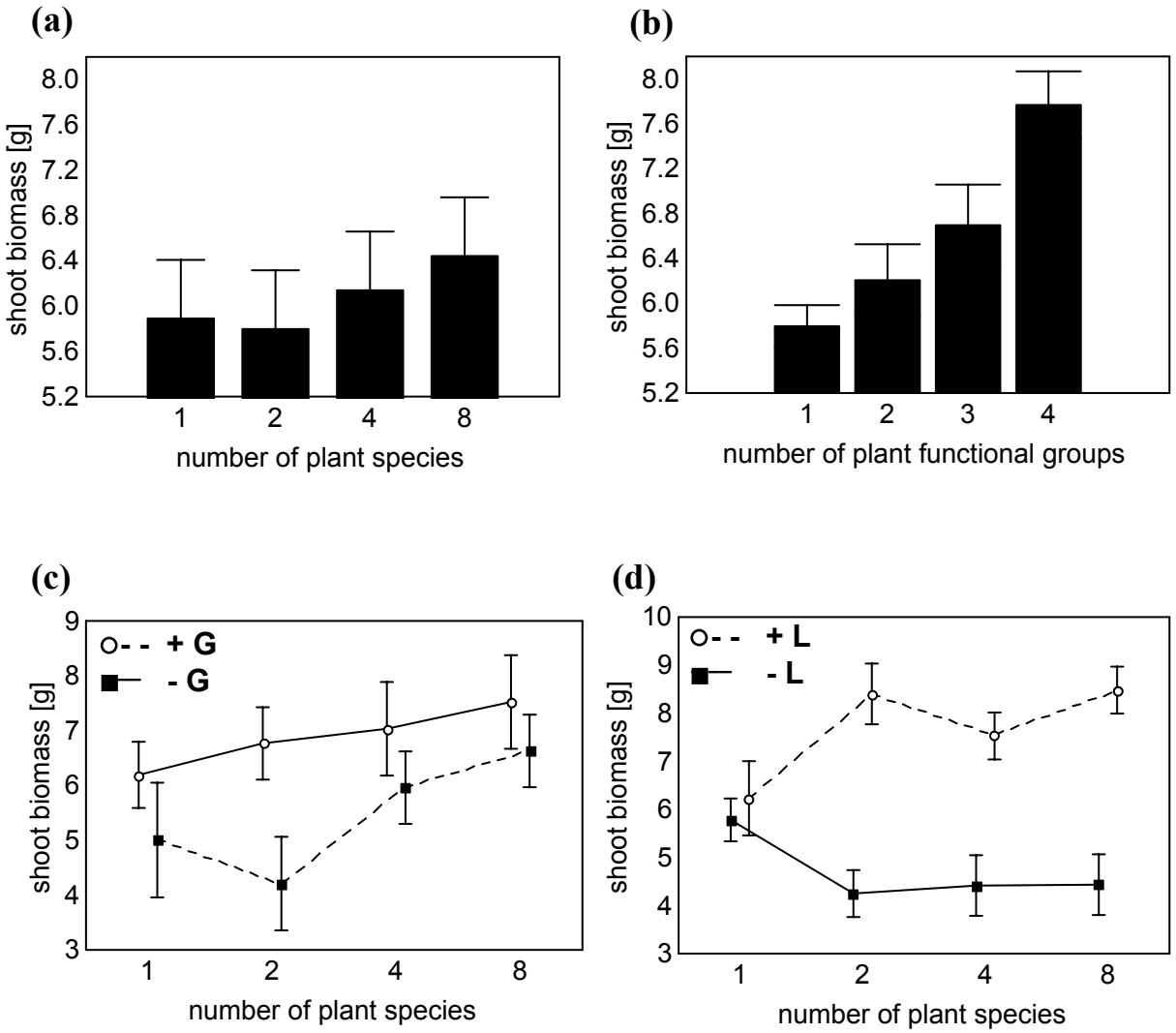

Figure 3.1 Effects of (a) plant species diversity, (b) plant functional group diversity, (c) presence of grasses and (d) presence of legumes on shoot biomass (g dry weight). For (c) and (d) dashed line indicates presence of grasses and legumes, respectively. Bars indicate ± SE.

Decomposers strongly increased shoot biomass, being at a maximum in the earthworm only treatment (Fig. 3.2a, Table 3.2). In order to exclude a sampling effect due to the presence of legumes we also analysed shoot biomass without legumes. Shoot biomass was generally lower without legumes and varied between 3.1 ± 0.3 g and 3.9 ± 0.3 g. Plant species and plant functional group diversity both negatively affected shoot biomass without legumes (Table 3.2).

Plant biomass was highest in the one species (4.3 ± 0.3 g) and one plant functional group (3.8 ± 0.2 g) mixtures, respectively. It decreased in the two species (3.3 ± 0.3 g) and two plant functional group (3.2 ± 0.3 g) mixtures, and increased again up to the eight species (3.5 ± 0.3 g) and four functional group (3.4 ± 0.4 g) mixtures, respectively. The effect of grasses and legumes on shoot biomass without legumes was opposite to their effect on total shoot biomass (Table 3.2): presence of grasses increased it from 3.2 ± 0.2 g to 4.0 ± 0.2 g and presence of legumes decreased it from 4.9 ± 0.1 g to 2.2 ± 0.2 g. The effect of tall and small herbs on shoot biomass without legumes was similar to that of shoot biomass including legumes (Table 3.2). Earthworms (+ 0.49 g) and collembolans (+ 0.31 g) also significantly increased shoot biomass without legumes (Table 3.2). The impact was most pronounced in the combined treatment with earthworms and collembolans (+0.8 g compared to the control treatment).

In addition, we calculated total shoot biomass and average shoot biomass per pot for each of the plant functional groups. Total shoot biomass of grasses per pot was decreased by small herbs (-2.1 g), tall herbs (-1.5 g), and increasing plant species and plant functional group diversity (from 5.0 ± 0.3 g in monocultures to 2.1 ± 0.2 g in eight species mixtures and from 4.6 ± 0.2 g in one plant functional group mixtures to 1.4 ± 0.2 g in four plant functional group mixtures, respectively; Table 3.5). The average shoot biomass of grasses per pot was increased by tall herbs (+0.1 g. Average shoot biomass of grasses was at a minimum in two species mixtures (0.5 ± 0.05 g) and two plant functional group mixtures (0.5 ± 0.05 g), respectively, while it was at a maximum in eight species (0.7 ± 0.04 g) and three plant functional group mixtures (0.7 ± 0.05 g), respectively (Table 3.5). Shoot biomass of legumes was decreased by small herbs (-1.5 g), and plant functional group diversity (from 7.8 ± 0.4 g to 4.3 ± 0.4 g). Also, plant species diversity affected shoot biomass of legumes with a maximum in two species mixtures (6.7 ± 0.5 g) and a minimum in four species mixtures (5.0 ± 0.4 g). In contrast, average shoot biomass of legumes per pot was increased by increasing plant species and plant functional group diversity, ranging from 0.7 ± 0.2 g to 2.0 ± 0.1 g and 1.0 ± 0.1 g to 2.2 ± 0.1 g, respectively.

Shoot biomass of tall herbs was decreased by grasses (-2.6 g), legumes (-2.0 g), and plant species (from 5.0 ± 0.3 g to 1.3 ± 0.2 g) and plant functional group diversity (from 4.6 ± 0.1 g to 0.6 ± 0.1 g). The average shoot biomass of tall herbs per pot was decreased by grasses (-0.2 g), small herbs (- 0.1 g), and increasing plant species (from 0.6 ± 0.05 g to 0.4 ± 0.03 g) and plant functional group diversity (from 0.6 ± 0.03 g to 0.3 ± 0.03 g).

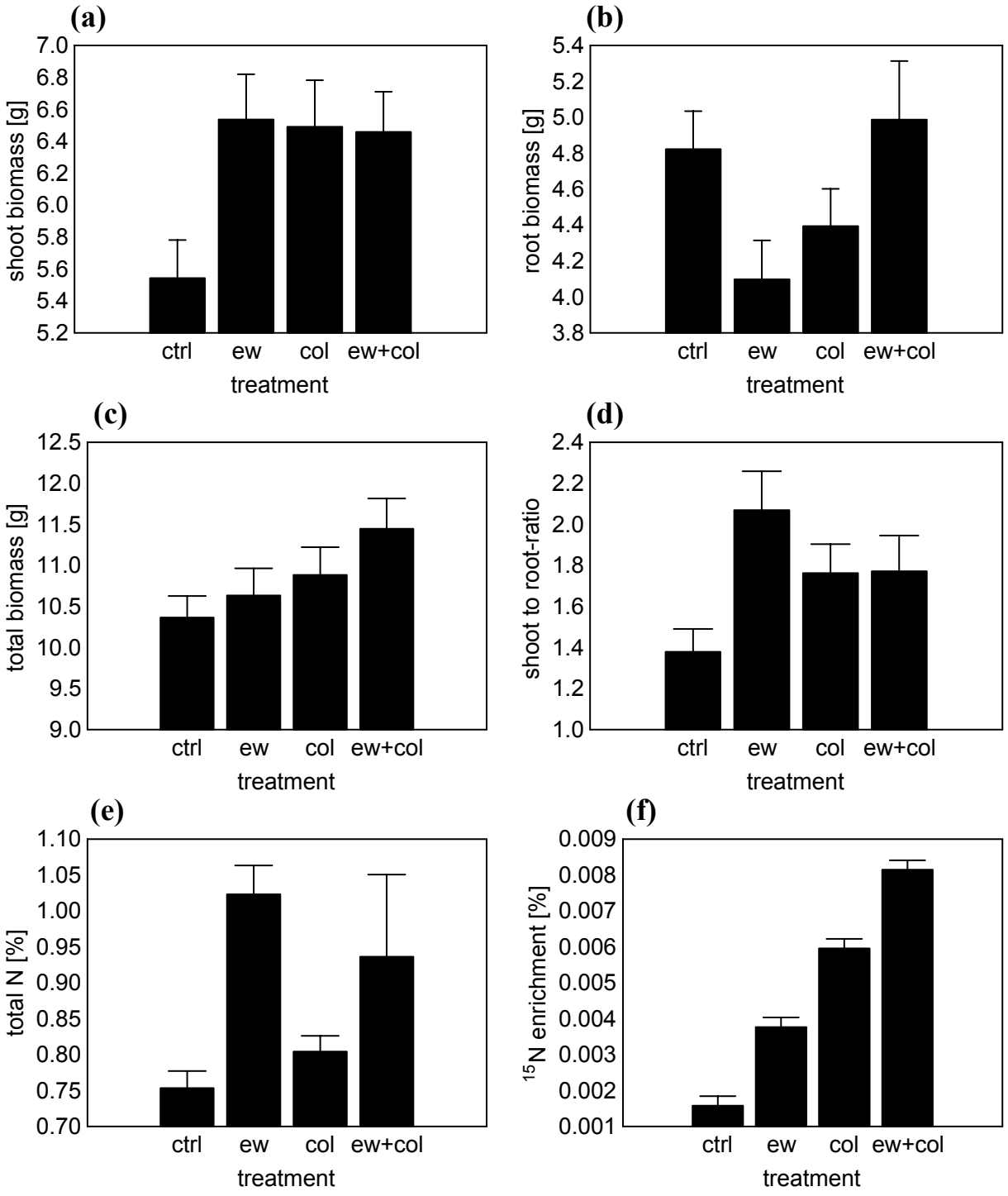

Figure 3.2 Effects of earthworms and collembolans on (a) shoot biomass (g dry weight), (b) root biomass (g dry weight), (c) total plant biomass (g dry weight), (d) shoot-to-root ratio, (e) total N content of *Festuca rubra* and (f) ^{15}N enrichment of *Festuca rubra*. Ctrl = control treatment, ew = earthworms only, col = collembolans only, ew+col = combined treatment with earthworms and collembolans. Bars indicate ± SE.

Shoot biomass of small herbs was decreased by tall herbs (- 2.8 g) and again by increasing plant species (from 7.3 ± 0.5 g to 2.3 ± 0.3 g) and plant functional group diversity (from 6.2 ± 0.3 g to 1.6 ± 0.3). The average shoot biomass of small herbs per pot was only affected by plant species diversity and plant functional group diversity. It varied between 1.0 ± 0.1 g in monocultures and 0.6 ± 0.07 g in four species mixtures. Mixtures with one, two and four plant functional groups showed merely the same average shoot biomass of small herbs (average 0.8 g), while mixtures with three plant functional groups decreased it (0.5 ± 0.09 g).

Earthworms increased total biomass of grasses per pot by 0.3 g (Table 3.5) and the average shoot biomass of grasses by 0.1 g (Table 3.4). Earthworms also increased shoot biomass of tall herbs (+0.3 g; Table 3.5). Collembolans increased average shoot biomass (+0.3 g) and total shoot biomass of tall herbs (+0.9 g; Table 3.5, 3.5). The effect on total shoot biomass of tall herbs was most pronounced in the combined treatment with earthworms and collembolans (no significant EW x COL interaction). Also, collembolans tended to increase pot biomass of grass shoots (Table 3.5). Earthworms and collembolans tended to increase shoot biomass of legumes per pot. However, the effect was less pronounced in the combined treatment (EW x COL interaction; Table 3.5). Biomass of small herbs was not affected by the presence of decomposers.

We also calculated the biomass per pot and the mean weight of individual plants per pot for the three plant species studied in more detail (*Festuca rubra*, *Onobrychis viciifolia*, *Plantago lanceolata*). Biomass of individual shoots of *Festuca rubra* increased with increasing plant species diversity from 0.65 ± 0.2 g to 1.65 ± 0.2 g ($F_{3,15} = 8.92$, $P = 0.001$) and from mixtures with one (0.7 ± 0.1 g) to mixtures with three plant functional groups (1.7 ± 0.2 g; $F_{3,15} = 9.44$, $P = 0.001$). In mixtures with four plant functional groups it decreased again to a level similar to one plant functional group mixtures (0.6 ± 0.2 g). Biomass of individual shoots of *Onobrychis viciifolia* increased by 0.3 g on average from mixtures with one to mixtures with four plant functional groups ($F_{2,21} = 5.76$, $P = 0.01$). Pot biomass of all three plant species studied was also affected by plant diversity. Increasing plant species diversity decreased pot biomass of *Festuca rubra* (from 5.2 ± 0.2 g in monocultures to 1.7 ± 0.2 g in eight species mixtures), *Onobrychis viciifolia* (from 5.6 ± 0.3 g to 0.5 ± 0.1 g) and *Plantago lanceolata* (from 6.0 ± 0.4 g to 1.3 ± 0.2 g). Plant functional group diversity decreased pot biomass of *Festuca rubra* (from 4.0 ± 0.4 g in one plant functional group mixtures to 1.3 ± 0.5 g in four plant functional group mixtures), *Onobrychis viciifolia* (from 2.2 ± 0.5 g to 1.0 ± 0.1g) with a

minimum (0.4 ± 0.1 g) in mixtures with three plant functional groups, and *Plantago lanceolata* (from 4.8 ± 0.4 g to 1.8 ± 0.3g).

Earthworms increased pot biomass of *Festuca rubra* from 2.5 ± 0.4 to 3.0 ± 0.4 ($F_{1,15}$ = 5.21, P = 0.038) and biomass of individual shoots of *F. rubra* in the one, two and eight species mixtures (significant EW x S interaction; $F_{3,6}$ = 8.93, P = 0.013), leading to an average increase of *F. rubra* shoots by 0.2 g. Earthworms also increased pot biomass of *Plantago lanceolata* in the one, two and four species mixtures, but slightly decreased it in pots with eight plant species (interaction EW x S; $F_{3,35}$ = 2.91, P = 0.048). On average, collembolans increased biomass of individual shoots of *Onobrychis viciifolia* by 0.2 g ($F_{1,21}$ = 9.14, P = 0.007). Both earthworms and collembolans affected the pot biomass of *O. viciifolia* and the effect varied with plant species diversity (significant EW x COL x S interaction; $F_{9,11}$ = 29.22, P < 0.0001).

Earthworms increased pot biomass of *Onobrychis viciifolia* in one, two and four species mixtures and very slightly decreased it in eight species mixtures, whereas collembolans very slightly increased pot biomass of *Onobrychis viciifolia* in four and eight species mixtures, but decreased it in two species mixtures. The negative effect of collembolans in two species mixtures was less pronounced when earthworms were present. The effect of collembolans on *Onobrychis viciifolia* monocultures also depended on the presence of earthworms. Generally the impact of decomposers on *Onobrychis viciifolia* was more pronounced in mixtures with lower diversity.

Total N concentrationt and ^{15}N enrichment of plants

Plant species and plant functional group diversity significantly affected ^{15}N incorporation but not the concentration of total N in the studied plant species. ^{15}N concentration in plant tissue of *F. rubra* increased with increasing plant species and plant functional group diversity. It varied between 0.004 ± 0.001% in monocultures and 0.006 ± 0.001% in eight species mixtures, and 0.004 ± 0.001% in one plant functional group mixtures and 0.005 ± 0.001% in four plant functional group mixtures, being at a maximum in mixtures three plant functional groups (0.006 ± 0.001%). ^{15}N enrichment of Onobrychis viciifolia was increased from monocultures (0.01 ± 0.002) to eight species mixtures (0.02 ± 0.002) and form one plant functional group mixtures (0.01 ± 0.001%) to four plant functional group mixtures (0.02 ± 0.002). ^{15}N enrichment was highest in shoot tissue of *Planago lanceolata* and it was only

affected by plant functional group diversity, being at a maximum in mixtures with two plant fuctional groups (0.07 ± 0.004).

In treatments with decomposers, total N concentration and ^{15}N enrichment of *Festuca rubra*, *Onobrychis viciifolia* and *Plantago lanceolata* was generally increased compared to the control treatment without earthworms and collembolans. Variations in total N and ^{15}N concentration were very similar in each of the three plant species studied; total N concentration was at a maximum in the earthworm only treatment and enrichment in ^{15}N in the combined treatment with earthworms and collembolans (Fig. 3.2 e, f). Total N concentration of *Festuca rubra* and *Plantago lanceolata* was significantly increased by earthworms with the effect being less pronounced when collembolans were present. The incorporation of ^{15}N into plant tissue of *Festuca rubra* and *Onobrychis viciifolia* was significantly increased by earthworms and collembolans, and their interaction. For *Plantago lanceolata* only collembolans significantly increased ^{15}N enrichment. The effects of earthworms ($F_{3,25} = 5.51$, $P = 0.005$; $F_{2,25} = 5.19$, $P = 0.013$) and collembolans ($F_{3,25} = 9.47$, $P = 0.0002$; $F_{2,25} = 3.81$, $P = 0.036$) on the ^{15}N enrichment of *Plantago lanceolata* varied with plant species diversity and plant functional group diversity, respectively. Earthworms increased ^{15}N incorporation in all but the one species and one functional group mixtures, respectively. Collembolans increased incorporation of ^{15}N in all but the four species mixtures. In addition, the effect of collembolans on incorporation of ^{15}N into pant tissue of *Festuca rubra* varied with plant functional group diversity and was at a maximum (0.008 ± 0.001%) in the mixtures with three plant functional groups (Table 3.3).

Root biomass

Root biomass varied between 4.1 ± 0.3 g and 5.0 ± 0.3 g. It decreased with increasing plant species but not plant functional group diversity (Table 3.2). Presence of grasses increased root biomass from 4.4 ± 0.2 g to 4.8 ± 0.2 g, whereas presence of small herbs generally decreased it from 4.9 ± 0.2 g to 4.2 ± 0.2 g (Table 3.2). However, the effect of small herbs varied with plant species diversity: small herbs strongly decreased root biomass in the one (- 1.8 g) and two species mixtures (-1.3 g), and slightly increased it in the four (+0.4 g) and eight species mixtures (+ 0.1 g). Presence of legumes and presence of tall herbs showed no overall effect on root biomass which was due to the variation of their impact with varying plant species diversity: legumes tended to increase root biomass in all (1: +0.7 g, 4: +0.6 g, 8: +0.1 g) but

the two species mixtures (-1.1 g), whereas tall herbs increased root biomass only in the two (+ 1.4 g) and eight species mixtures (+ 0.3 g) (Table 3.2).

Root biomass decreased in the collembolan only treatment (- 0.4 g), and even stronger in the earthworm only treatment (-0.7 g). However, it increased on average by 0.2 g when both animal groups were present (significant EW x COL interaction; Fig. 3.2b, Table 3.2).

Total plant biomass

Plant functional group diversity but not plant species diversity increased total plant biomass, with a maximum in the four plant functional group mixtures, differing significantly from total biomass of the one and two plant functional group mixtures (Table 3.2). Overall, presence of legumes increased total plant biomass, whereas presence of grasses and presence of tall herbs significantly decreased it, but again, the effect of the plant functional groups differed between plant species diversity levels: the positive effect of legumes was strongest in the eight species mixtures and less pronounced in monocultures. Grasses increased total plant biomass in monocultures, but decreased it in two, four and eight species mixtures, whereas tall herbs decreased total plant biomass in one, two and four species mixtures, but slightly increased it in the eight species mixtures.

Both decomposer invertebrate groups positively influenced total plant biomass per pot, being at a maximum in the combined treatment with earthworms and collembolans (Fig. 3.2c, Table 3.2), but only presence of collembolans significantly increased total plant biomass (Table 3.2).

Shoot-to-root ratio

Plant species and plant functional group diversity altered the shoot-to-root ratio (Table 3.2). It was at a maximum in monocultures (1.9 ± 0.2) and at a minimum in two species mixtures (1.5 ± 0.2), and increased with plant functional group diversity from 1.7 ± 0.1 to 2.1 ± 0.1. Also, presence of each of the four plant functional groups affected the shoot-to-root ratio: presence of grasses (- 0.5) and presence of tall herbs (- 0.3) decreased it, whereas presence of legumes (+0.8) and presence of small herbs (+0.7) increased it (Table 3.2). Again, the effect of legumes and small herbs varied with plant species diversity. The effect of legumes was most pronounced in the two species mixtures (shhot to root ratio 2.1 ± 0.3) and was slightly

negative in the monocultures, wherease the impact of small herbs was strongest in monocultures (3.4 ±0.3).

Earthworms increased the shoot-to-root ratio, but the effect was less pronounced when collembolans were present in the same pots (Fig. 3.2d). In addition, collembolans tended to increase plant shoot-to-root ratio.

Table 3.2 ANOVA table of F-values on the effects of earthworms (EW), collembolans (COL), number of plant species (S), number of plant functional groups (FG) and presence of legumes (L), grasses (G), small herbs (Sh) and tall herbs (Th) on total plant biomass, total shoot biomass, shoot biomass without legumes, root biomass and shoot to root ratio.

	Total biomass	**Shoot biomass**	**Shoot biomass without legumes**	**Root biomass**	**Shoot-to-root ratio**
EW	$F_{1,243}$ = 2.06	**$F_{1,243}$ = 8.24****	**$F_{1,211}$ = 8.36****	$F_{1,243}$ = 0.07	**$F_{1,243}$ = 5.20***
COL	**$F_{1,243}$ = 5.30***	**$F_{1,243}$ = 7.49****	**$F_{1,211}$ = 6.24***	$F_{1,243}$ = 0.83	$F_{1,243}$ = 0.40
EW x COL	$F_{1,243}$ = 0.26	**$F_{1,243}$ = 6.44***	$F_{1,211}$ = 1.64	**$F_{1,243}$ = 6.79****	**$F_{1,243}$ = 10.15****
FG	**$F_{3,243}$ = 4.93****	**$F_{3,243}$ = 18.44*****	**$F_{3,211}$ = 39.01*****	$F_{3,243}$ = 0.48	**$F_{3,243}$ = 4.64****
FG linear	**$F_{1,252}$ = 9.10****	**$F_{1,252}$ = 23.10*****	**$F_{1,220}$ = 41.22*****	$F_{1,252}$ = 0.78	**$F_{1,252}$ = 8.78****
FG deviation	$F_{2,252}$ = 0.76	$F_{2,252}$ = 0.95	**$F_{2,220}$ = 18.33*****	$F_{2,252}$ = 0.28	$F_{2,252}$ = 0.68
S	$F_{3,243}$ = 0.64	**$F_{3,243}$ = 9.66*****	**$F_{3,211}$ = 30.47*****	$F_{3,243}$ = 2.55	**$F_{3,243}$ = 5.08****
S log_2 linear	$F_{1,252}$ = 0.81	**$F_{1,252}$ = 7.78****	**$F_{1,220}$ = 32.06*****	**$F_{1,252}$ = 4.16***	$F_{1,252}$ = 3.51
S deviation	$F_{2,252}$ = 0.26	$F_{2,252}$ = 2.36	**$F_{2,220}$ = 12.21*****	$F_{2,252}$ = 1.58	**$F_{2,252}$ = 3.81***
L	**$F_{1,243}$ = 88.85*****	**$F_{1,243}$ = 232.49*****	**$F_{1,211}$ = 71.85*****	$F_{1,243}$ = 0.01	**$F_{1,243}$ = 46.77*****
L x FG	$F_{2,238}$ = 2.36	**$F_{2,238}$ = 6.58****	$F_{1,208}$ = 2.18	$F_{2,238}$ = 0.77	$F_{2,238}$ = 2.18
L x S	**$F_{3,238}$ = 5.90*****	**$F_{3,238}$ = 24.35*****	$F_{2,208}$ = 1.15	$F_{3,238}$ = 2.64	**$F_{3,238}$ = 9.72*****
G	**$F_{1,243}$ = 21.37*****	**$F_{1,243}$ = 130.15*****	**$F_{1,211}$ = 17.32*****	**$F_{1,243}$ = 6.98****	**$F_{1,243}$ = 48.74*****
G x FG	**$F_{2,238}$ = 12.88*****	**$F_{2,238}$ = 17.67*****	**$F_{2,206}$ = 28.63*****	$F_{2,238}$ = 2.64	$F_{2,238}$ = 2.93
G x S	**$F_{3,238}$ = 6.07*****	**$F_{3,238}$ = 4.24****	**$F_{3,206}$ = 12.30*****	$F_{3,238}$ = 2.49	$F_{3,238}$ = 0.62
Sh	$F_{1,243}$ = 0.01	**$F_{1,243}$ = 12.29*****	**$F_{1,211}$ = 19.90*****	**$F_{1,243}$ = 7.03****	**$F_{1,243}$ = 14.65*****
Sh x FG	$F_{2,238}$ = 2.22	$F_{2,238}$ = 1.47	$F_{2,206}$ = 0.65	$F_{2,238}$ = 0.42	$F_{2,238}$ = 0.09
Sh x S	**$F_{3,238}$ = 2.74***	**$F_{3,238}$ = 4.16****	**$F_{3,206}$ = 3.34***	**$F_{3,238}$ = 3.37***	**$F_{3,238}$ = 4.50****
Th	**$F_{1,243}$ = 23.17*****	**$F_{1,243}$ = 53.94*****	**$F_{1,211}$ = 6.67***	$F_{1,243}$ = 0.01	**$F_{1,243}$ = 13.58*****
Th x FG	$F_{2,238}$ = 1.19	$F_{2,238}$ = 1.56	$F_{2,206}$ = 0.13	$F_{2,238}$ = 0.90	$F_{2,238}$ = 0.90
Th x S	**$F_{3,238}$ = 3.60**	$F_{3,238}$ = 2.34	$F_{3,206}$ = 0.80	**$F_{3, 238}$ = 4.19****	$F_{3,238}$ = 2.12

Table 3.3 ANOVA table of F-values on the effects of earthworms (EW), collembolans (COL), number of plant species (S) and number of plant functional groups (FG) on total N and ^{15}N concentration of *Festuca rubra*, *Onobrychis viciifolia* and *Plantago lanceolata*.

	Festuca rubra		***Onobrychis viciifolia***		***Plantago lanceolata***	
	Total N content	**^{15}N enrichment**	**Total N content**	**^{15}N enrichment**	**Total N content**	**^{15}N enrichment**
EW	**$F_{1,15} = 9.58$****	**$F_{1,15} = 154.44$*****	$F_{1,22} = 2.01$	**$F_{1,22} = 1846.90$*****	**$F_{1,35} = 8.92$****	$F_{1,25} = 3.62$
COL	$F_{1,15} = 0.17$	**$F_{1,15} = 522.19$*****	$F_{1,22} = 0.48$	**$F_{1,22} = 8200.81$*****	$F_{1,35} = 1.10$	**$F_{1,25} = 5.86$***
EW x COL	$F_{1,15} = 1.87$	**$F_{1,15} = 24.25$*****	$F_{1,22} = 0.05$	**$F_{1,22} = 6.63$***	$F_{1,35} = 3.65$	$F_{1,25} = 0.65$
FG	$F_{3,15} = 1.65$	**$F_{3,15} = 16.9$*****	$F_{2,22} = 1.63$	**$F_{2,22} = 180.57$*****	$F_{2,35} = 1.57$	**$F_{2,25} = 4.99$***
FG linear	$F_{1,.20} = 2.73$	$F_{1,20} = 1.14$	$F_{1,28} = 4.05$	$F_{1,28} = 1.52$	$F_{1,41} = 0.03$	$F_{1,41} = 0.01$
FG deviation	$F_{2.20} = 0.45$	$F_{2.20} = 0.13$	$F_{1,28} = 0.02$	$F_{1,28} = 0.02$	$F_{1,41} = 2.57$	**$F_{1,41} = 4.31$***
EW x FG	$F_{3,9} = 0.45$	$F_{3,9} = 3.51$	$F_{2,18} = 0.27$	$F_{2,18} = 1.16$	$F_{2,31} = 0.45$	$F_{2,31} = 2.93$
COL x FG	$F_{3,9} = 1.14$	**$F_{3,9} = 8.98$****	$F_{2,18} = 0.52$	$F_{2,18} = 1.05$	$F_{2,31} = 1.98$	$F_{2,31} = 2.15$
S	$F_{3,15} = 1.29$	**$F_{3,15} = 17.16$*****	$F_{3,22} = 0.93$	**$F_{3,22} = 180.15$*****	$F_{3,35} = 0.65$	$F_{3,25} = 1.34$
S log_2 linear	$F_{1,20} = 0.78$	$F_{1,20} = 1.42$	$F_{1,27} = 2.35$	$F_{1,27} = 1.91$	$F_{1,40} = 0.71$	$F_{1,40} = 1.50$
S deviation	$F_{2.20} = 0.96$	$F_{2,20} = 0.01$	$F_{2,27} = 0.39$	$F_{2,27} = 0.08$	$F_{2,40} = 0.41$	$F_{2,40} = 0.05$
EW x S	$F_{3,9} = 1.26$	**$F_{3,9} = 4.16$***	$F_{3,16} = 1.23$	$F_{3,16} = 0.04$	$F_{3,29} = 0.82$	**$F_{3,29} = 5.70$***
COL x S	$F_{3,9} = 1.69$	**$F_{3,9} = 10.59$****	$F_{3,16} = 0.54$	$F_{3,16} = 0.01$	$F_{3,29} = 0.50$	**$F_{3,29} = 9.79$***

*****, P < 0.001; **, P < 0.01; *, P < 0.05**

Table 3.4 ANOVA table of F-values on the effects of earthworms (EW), collembolans (COL), number of plant species (S), number of plant functional groups (FG) and presence of legumes (L), grasses (G), small herbs (Sh) and tall herbs (Th) on the mean individual weight of the plant functional groups.

	Mean individual weight			
	Grasses	**Legumes**	**Tall herbs**	**Small herbs**
EW	**$F_{1,108} = 5.03$***	$F_{1,108} = 0.44$	$F_{1,108} = 1.11$	$F_{1,108} = 1.38$
COL	$F_{1,108} = 1.39$	$F_{1,108} = 0.09$	**$F_{1,108} = 7.63$****	$F_{1,108} = 0.02$
EW x COL	$F_{1,108} = 1.56$	$F_{1,108} = 1.61$	$F_{1,108} = 0.03$	$F_{1,108} = 0.38$
FG	**$F_{3,108} = 6.37$*****	**$F_{3,108} = 13.21$*****	**$F_{3,108} = 13.32$*****	**$F_{3,108} = 2.9$*8**
FG linear	**$F_{1,116} = 3.95$***	**$F_{1,116} = 37.32$*****	**$F_{1,116} = 28.19$*****	$F_{1,116} = 0.62$
FG deviation	**$F_{2,116} = 6.57$****	$F_{2,116} = 0.86$	**$F_{2,116} = 3.58$***	**$F_{2,116} = 4.03$***
S	**$F_{3,108} = 3.04$***	**$F_{3,108} = 10.63$*****	**$F_{3,108} = 9.53$*****	**$F_{3,108} = 2.91$***
S $\log_2$ linear	$F_{1,116} = 2.94$	**$F_{1,116} = 28.42$*****	**$F_{1,116} = 17.49$*****	$F_{1,116} = 2.38$
S deviation	$F_{2,116} = 2.32$	$F_{2,116} = 0.52$	$F_{2,116} = 2.88$	$F_{2,116} = 3.03$
L	$F_{1,108} = 2.77$	-	$F_{1,108} = 2.15$	$F_{1,108} = 0.10$
G	-	$F_{1,108} = 0.12$	**$F_{1,108} = 7.83$****	$F_{1,108} = 0.74$
Sh	$F_{1,108} = 2.01$	$F_{1,108} = 1.91$	**$F_{1,108} = 4.44$***	-
Th	**$F_{1,108} = 6.10$***	$F_{1,108} = 1.35$	-	$F_{1,108} = 3.46$

*****, P < 0.001; **, P < 0.01; *, P < 0.05**

Table 3.5 ANOVA table of F-values on the effects of earthworms (EW), collembolans (COL), number of plant species (S), number of plant functional groups (FG) and presence of legumes (L), grasses (G), small herbs (Sh) and tall herbs (Th) on pot biomass of the plant functional groups.

	Shoot biomass per pot			
	Grasses	**Legumes**	**Tall herbs**	**Small herbs**
EW	**$F_{1,108}$ = 4.94***	$F_{1,108}$ = 0.01	**$F_{1,108}$ = 5.83***	$F_{1,111}$ = 2.60
COL	$F_{1,108}$ = 3.44	$F_{1,108}$ = 0.48	**$F_{1,108}$ = 7.84****	$F_{1,111}$ = 0.41
EW x COL	$F_{1,108}$ = 2.74	$F_{1,108}$ = 3.75	$F_{1,108}$ = 0.02	$F_{1,111}$ = 0.18
FG	**$F_{3,108}$ = 99.92****	**$F_{3,108}$ = 20.28*****	**$F_{3,108}$ = 291.66*****	**$F_{3,111}$ = 63.98*****
FG linear	**$F_{1,116}$ = 191.04****	**$F_{1,116}$ = 45.26*****	**$F_{1,116}$ = 517.26*****	**$F_{1,119}$ = 122.11*****
FG deviation	**$F_{2,116}$ = 27.30****	$F_{2,116}$ = 2.73	**$F_{2,116}$ = 60.41*****	**$F_{2,119}$ = 21.23*****
S	**$F_{3,108}$ = 67.36****	**$F_{3,108}$ = 4.15*****	**$F_{3,108}$ = 186.01*****	**$F_{3,111}$ = 51.83*****
S log_2 linear	**$F_{1,116}$ = 67.69*****	$F_{1,116}$ = 3.03	**$F_{1,116}$ = 102.97*****	**$F_{1,119}$ = 70.73*****
S deviation	**$F_{2,116}$ = 15.14*****	$F_{2,116}$ = 2.34	**$F_{2,116}$ = 16.34*****	**$F_{2,119}$ = 17.51*****
L	$F_{1,108}$ = 1.62	-	**$F_{1,108}$ = 17.33*****	$F_{1,111}$ = 0.26
G	-	$F_{1,108}$ = 0.26	**$F_{1,108}$ = 19.15*****	$F_{1,111}$ = 0.03
Sh	**$F_{1,108}$ = 6.32***	**$F_{1,108}$ = 5.04*****	$F_{1,108}$ = 2.10	-
Th	**$F_{1,108}$ = 11.02****	$F_{1,108}$ = 3.70	-	**$F_{1,111}$ = 7.64****

*****, P < 0.001; **, P < 0.01; *, P < 0.05**

3.5 Discussion

Plant diversity

Recent studies documented that net primary productivity (NPP) and nutrient retention in ecosystems increase as the number of plant species increases (Hooper & Vitousek 1997, Hector et al. 1999, Spehn et al. 2005). Major mechanisms responsible for the increase in productivity with diversity are the complementary use of resources by plants, facilitative interactions, and "sampling effects", resulting from the greater probability of including dominant and highly productive species or combinations of species (Spehn et al. 2005). In the present study, plant species and plant functional group diversity generally strongly influenced plant performance, especially aboveground productivity. Overall, shoot biomass increased log-linearly with plant species and linearly with plant functional group diversity, which parallele an increase in ^{15}N enrichment in shoot tissue of three selected plant species. In addition to plant richness per se, further variance was explained by plant species composition, i.e. the identity of functional groups. The specific traits of the different plant functional groups influenced plant productivity per pot differently and most of the effects varied with plant species diversity. For example, small and tall herbs did best in monocultures and were negatively influenced by increasing plant species and plant functional group diversity, while grasses and legumes benefited from increasing plant species and plant functional group diversity. As suggested previously (Tilman et al. 1997, Diaz & Cabido 2001) functional traits of plants species presumably are more important than plant species and plant functional group diversity per se.

When excluding shoot biomass of legumes from total shoot biomass, the positive influence of plant diversity disappeared, indicating a "sampling effect" due to the highly productive legumes. For example, there is evidence that non-legume plant species benefit from symbiotic nitrogen fixation by legumes (Spehn et al. 2002).

Root biomass was little affected by plant diversity. It decreased log linearly with increasing plant species diversity which was paralleled by a log-linear decrease in microbial respiration ($F_{1,247} = 5.85$, $P = 0.0163$). Small herbs had an overall negative effect on root biomass, and tall herbs decreased root biomass in the one and four species mixtures. Herbs are known to have allelopathic effects on neighbouring plants. For example, *Centaurea* species were suspected of being allelopathic 40 years ago (Fletcher & Renney 1963), and Bais et al. (2002) recently indeed found phytotoxic chemicals in root exudates of *Centaurea* species. Since

small herbs and tall herbs also negatively influenced pot biomass of grasses, and pot biomass of legumes they might have inhibited nutrient uptake of neighbouring plants. For example, an allelopathic agent against clover might directly attack the symbiotic bacteria and destroy the plant's source of nitrogen. In our study the negative impact of tall herbs on root biomass, and on shoot biomass of grasses and legumes, correlated with an increased microbial respiration by ca. 5% ($F_{1,237}$ = 6.95, P = 0.009) in presence of tall herbs. Root exudates are readily assimilated for microorganisms. They represent an easily available source of C and might favour fast growing microbes (Baudoin 2003). Indeed, root exudates were found to increase respiration of bacteria (Kozdrój 2000). In the present study the increased microbial respiration might therefore indicate increased abundance of root exudates. However, the mechanisms implied by the negative effect of tall herbs are beyond interpretation of this study.

Earthworms and collembolans

Soil microorganisms dominate mineralisation processes and compete with plants for nutrients (Kaye & Hart 1997, Hodge et al. 2000). Soil invertebrates affect the soil microbial community and functioning directly by grazing but also indirectly by changing nutrient availability and soil structure; both direct and indirect effects are known to affect plant performance and ecosystem processes (Scheu 2001, Brown & Doube 2004, Bonkowski 2004). Results of the present study suggest that both earthworms and collembolans increase plant performance through enhanced nutrient mineralisation and an accompanying increase in plant nutrient acquisition. Incorporation of surface litter material into the soil was highest in earthworm treatments and less pronounced when only collembolans were present. Despite their differential effects on litter dynamics both decomposer groups had a similar positive influence on aboveground plant productivity suggesting that their effects were based on different mechanisms. Consistent with previous experiments (Scheu et al. 1999, Schmidt and Curry 1999, Kreuzer et al. 2004), earthworms generally enhanced plant growth, in particular that of grasses not that of legumes. Earthworms increased grass biomass per pot and the average biomass of grass shoots, and also tall herb biomass per pot, but not average biomass of shoots of tall herbs, suggesting that in contrast to grasses earthworms stimulated the growth of a single or a few, but not of all tall herb species. Presumably, plant species more independent of soil N respond less since earthworms alter plant growth by increasing N mineralisation.

Furthermore, there is evidence that legumes are less efficient in exploiting soil N than e.g. grasses (Kang 1988).

For collembolans, beneficial effects on plant performance also have been document (Scheu et al. 1999, Kreuzer et al. 2004). In contrast to earthworms, collembolans influence plant growth via feeding on fungi. However, the mechanisms for collembola-mediated changes in plant growth still are little understood. Gange (2000) and Lussenhop & BassiriRad (2005) documented that the effect of collembola on plant growth depend on the density of collembola. Moderate grazing on mycorrhizal fungi stimulates the activity of fungi and therefore enhances plant growth (Lussenhop 1996). In our study increased shoot biomass, the accompanying increase in the shoot-to-root ratio and the increased ^{15}N uptake by the three plant species studied support the conclusion of Bardgett & Chan (1999) and Gange (2000) that collembolans stimulate N mineralisation, as do earthworms.

The effect of earthworms on plant growth presumably was due to an earthworm-mediated increase in nutrients available to plants as suggested by increased N concentrations in shoots of *Festuca rubra* and *Plantago lanceolata*. Compared to earthworms the effect of collembolans on shoot biomass of non-legumes was less pronounced. This is surprising considering that collembolans increased total plant biomass per pot and average individual shoot biomass of tall herbs whereas earthworms did not affect biomass of tall herbs. The slight increase in shoot biomass of legumes in the collembolan treatment and the strong ^{15}N enrichment in shoot tissue of *Onobrychis viciifolia* supports the conclusion of Lussenhop (1993) that collembolans affect N acquisition of legumes by altering nodule occupancy. However, despite legumes appear to benefit from collembolans, the total number of collembolans in presence of legumes was significantly reduced by 23% ($F_{1,94} = 6.44$, $P = 0.0128$) suggesting that they in turn suffer from thje presence of legumes.

Due to variable effects of decomposers on plant species neither the effect of earthworms nor that of collembolans significantly varied with plant functional group diversity. The enhanced total amount of plant N in the earthworm treatment seems to have been responsible for the increase in pot biomass of *Plantago lanceolata*, while the driving force of the impact of earthworms and collembolans on pot biomass of *Onobrychis viciifolia* is still not clear. Both decomposer groups and their interaction very strongly increased ^{15}N uptake of *Onobrychis viciifolia* but not its total amount of N.

Plants not only responded with increasing shoot biomass to the presence of decomposers but also by changing plant resource allocation. Compared to roots earthworms disproportionately increased shoot biomass, resulting in an increased shoot to root-ratio. The reduction in root biomass in presence of each of the decomposer groups presumably reflects increased nutrient availability. Reduced root biomass in presence of collembolans has been observed in a number of studies (Larsen & Jakobsen 1996, Scheu et al. 1999, Bardgett & Chan 1999, Kreuzer et al. 2004) and has been ascribed to a collembola-mediated increase in plant nutrient uptake (Lussenhop & BassiriRad 2005). Reviewing published data Scheu (2003) reported that the effect of earthworms on root biomass is inconsistent; it was increased in most studies but in a number of studies it was reduced. Apart from enhanced nutrient mineralisation, the reduction in root biomass in presence of earthworms also may have resulted from reduced competition for nutrients between microbes and plant roots; indeed, in our experiment microbial biomass and respiration was significantly reduced in earthworm treatments.

Despite both decomposer groups separaretely reduced root biomass, it was increased in the combined treatment with earthworms and collembolans proving that both decomposer groups interacted in affecting plant growth. The mechanisms responsible for this interaction remain unclear. Potentially, presence of both decomposer groups stimulated the exploitation of nutrients by plants thereby increasing root biomass. In presence of legumes the tissue N concentration of *A. caliginosa* was independent of the presence of collembolans, but the amount of ^{15}N in *A. caliginosa* tissue decreased when collembolans were also present. This indicates that in presence of collembolans earthworms incorporated more N derived from atmospheric fixation by legumes. Thus, earthworms competed with neighbouring non-legume plant roots for the symbiotically fixed N, and likely also increased competition among plant roots. The short-term transfer of N from legumes to non-legume plants can occur directly through mycorrhizal fungi interconnecting the root systems of donor and receiver plants (Paynel et al. 2001). Collembolans might play a key role for this system. Since they graze on fungal hyphae, they were presumably able to interrupt the N transfer, resulting in a release of N into the soil.

Also, earthworms and collembolans increased total plant biomass with a maximum in the combined treatment, but only presence of collembolans lead to a significant increase. Since both groups affected shoot biomass in a similar way, the linear increase in total biomass and

the effect on the shoot-to-root ratio were due to the pronounced differences in root biomass in the different decomposer treatments.

The complementary effects of earthworms and collembolans on total plant biomass and ^{15}N enrichment presumably resulted from additivity of the effects of each of the decomposer groups. In other cases, complex interactions between earthworms and collembolans need to be addressed.

In this study we used different species of the two very different decomposer soil invertebrate groups studied, i.e. earthworms and collembolans, to cover a wide range of potential effects of decomposers on plant performance, and to analyse whether functional traits of soil decomposer animals matter. In the future the role of diversity of decomposer species within functional groups of decomposer animals needs to be investigated in order to understand functional redundancy/complementarity among decomposer animal species.

In conclusion, consistent with previous studies in the field results of our laboratory study suggest that functional traits of individual plant species are more important for plant productivity than plant species and plant functional group diversity per se. Also, consistent with earlier findings the results suggest that plant diversity for plant community performance is less important than the diversity of plant functional groups. Furthermore, different plant functional groups reacted differently to decomposer presence. This suggests that effects of the loss of plant diversity on ecosystem processes depend on the identity of species lost. Also, our data show that earthworms and collembolans generally affected above- and belowground plant performance. However, the effect varied with plant functional group identity and tended to vary with plant species diversity. Our results underline the important role of earthworms and collembolans as decomposers for plant nutrient uptake and primary production of grassland plant species. Both the increased shoot biomass and the reduced root biomass suggest a higher N mobilisation in decomposer treatments. In addition to their individual effects, earthworms and collembolans interacted in affecting total plant biomass and ^{15}N enrichments of shoots and root biomass. This shows that plants differentially respond to the presence of different decomposer animal groups. Both the non-uniform and complementary effects of earthworms and collembolans on plant performance indicate that functional diversity of soil invertebrates is important for the functioning of the aboveground system.

Chapter 4

Earthworms (*Lumbricus terrestris*) affect plant seedling recruitment and microhabitat heterogeneity

4.1 Abstract

The effects of the anecic earthworm *Lumbricus terrestris* L. on plant seedling recruitment and aggregation were investigated in a microcosm greenhouse experiment by varying plant functional groups (grasses, legumes, herbs), seed size (small and large), plant species diversity (1, 3, 6) and plant functional group diversity (1, 3).

Generally, earthworms quickly buried seeds irrespective of size and species. Secondary seed dispersal (Phase II dispersal) by earthworms affected seedling recruitment through selective promotion or repression depending on seed size and plant functional group, and affected plant community composition. Although in general recruitment of seedlings was lower in presence of *L. terrestris*, the recruited seedlings benefited from establishing in the vicinity of earthworm burrows. Plant species and functional group diversity little affected seedling recruitment. The established seedlings were strongly associated with the earthworm burrows and the strong aggregation of plants in the vicinity of earthworm burrows resulted in plant communities with a more heterogeneous small-scale architecture.

In conclusion, seed dispersal, seed burial, seedling recruitment and the spatial distribution of seedlings of plant species of different functional groups and with a wide range of seed size is strongly affected by *L. terrestris* and this likely affects plant community composition.

4.2 Introduction

Species diversity of plant communities is the result of the dynamics in plant mortality and seedling establishment of new arrivals in the regeneration niche, *sensu* Grub (1977). Understanding the mechanisms that drive the establishment of seedlings therefore is essential for understanding plant community establishment and maintenance. The number and size of seeds, and plant traits affecting seed dispersal are major factors driving seedling establishment. However, the establishment of seedlings also strongly depends on local processes, such as small scale disturbances (Grub 1977). There is evidence that after Phase I dispersal of seeds, which includes the displacement of seeds form the parent to the soil surface, earthworms play an important role in Phase II dispersal, i.e. the subsequent displacement of seeds on the soil surface or burial into the soil (Grant 1983, van der Reest & Rogaar 1988, Willems & Huijsmans 1994, Thompson, Green & Jewels 1994, Decaens et al. 2003). Selective ingestion and digestion of seeds (McRill & Sagar 1973, Shumway & Koide 1994), downward or upward seed transport (Hurka & Haase 1982, Grant 1983), acceleration (Ayanlaja et al. 2001) or delaying of seed germination (Grant 1983, Decaens et al. 2001) are the main mechanisms by which earthworms affect establishment of seedlings. Most studies on earthworm – seed interrelationships concentrated on the effects of earthworms on the soil seed bank, vertical dispersion and viability of seeds in the seed bank; only little is known on how earthworms may influence seedling recruitment and the composition of plant communities (Grant 1983, Pierce, Roggero & Tipping 1994, Willems & Huijsmans 1994).

Large surface feeding anecic earthworms, such as *Lumbricus terrestris* L. (Lumbricidae), are a dominant component of decomposer communities in virtually all non-acidic agricultural and forest ecosystems including pastures and meadows of the temperate zone. Furthermore, *L. terrestris* is a peregrine species which has been spread with European agricultural practices virtually all over the world including into pristine ecosystems previously devoid of earthworm species (Bohlen et al. 2004). Earthworms, in particular anecic species, function as ecosystem engineers modifying the physical structure of soils by changing soil aggregation, soil porosity and the distribution and abundance of microorganisms and other soil invertebrates (Wickenbrock & Heisler 1997, Maraun et al. 1999, Tiunov & Scheu 1999). Modification of the physical structure of soil by creating and modifying microhabitats functions as a small-scale disturbance which likely affects plant recruitment and therefore

potentially plant community structure (Connell 1978, Fox 1979). Furthermore, earthworm casts and burrows might be an important regeneration niche for plant seedlings (Crawley 1992, Kassen et al. 2000).

We performed a microcosm greenhouse experiment to evaluate (1) if *L. terrestris* affects seedling recruitment and establishment of plant species through promotion and/or repression of certain species, (2) if the effect of *L. terrestris* on plant recruitment varies with seed size and seed functional group identity (grasses, herbs, legumes), and (3) if spatial distribution of the recruited plant seedlings is affected by *L. terrestris*.

4.3 Material and methods

Experimental setup

The experiment was set up in microcosms consisting of PVC tubes (inner diameter 16 cm, height 38 cm) covered by a 1 mm mesh at the bottom to prevent *L. terrestris* from escaping but allow drainage of water. The soil (pH 8.1, carbon content 4.6%, C/N ratio 15.7) was taken from the southeastern edge of The Jena Experiment, a large biodiversity field experiment (Thuringia, Germany; Roscher et al. 2004). A total of 90 microcosms were filled with 5.5 kg of sieved (1 cm) and homogenized soil and placed in a temperature controlled greenhouse at a day/night regime of 16/8 h and 20/16 ± 2ºC. Before starting the experiment the microcosms were watered regularly for a month and germinating weeds (unwanted plants form the seedbank) were removed. One gram of dried litter (2.53% N, C/N ratio 17.3, dried at 60ºC and cut into pieces about 3 cm in length) collected near the Jena Experiment study site consisting mainly of grass leaves was placed on top of the soil prior to the addition of earthworms and seeds to simulate natural conditions. Two adult *L. terrestris* (average fresh weight 4.2 ± 0.94 g) were introduced in half of the microcosms creating two treatments (Control and Earthworms). Within each treatment seeds of six plant species consisting of two seed size classes (small and large; Table 4.1) and three functional groups (grasses, herbs and legumes) were sown. The species were selected from the species pool of the Jena Experiment (Roscher et al. 2004) and sown as monocultures and two mixtures with three species (one mixture with the small seed size species and one mixture with the large seed size species), in a factorial and fully randomized design with five replicates per species composition (Table 4.1). An additional mixture with all six species was also set up to check for the effects of species diversity.

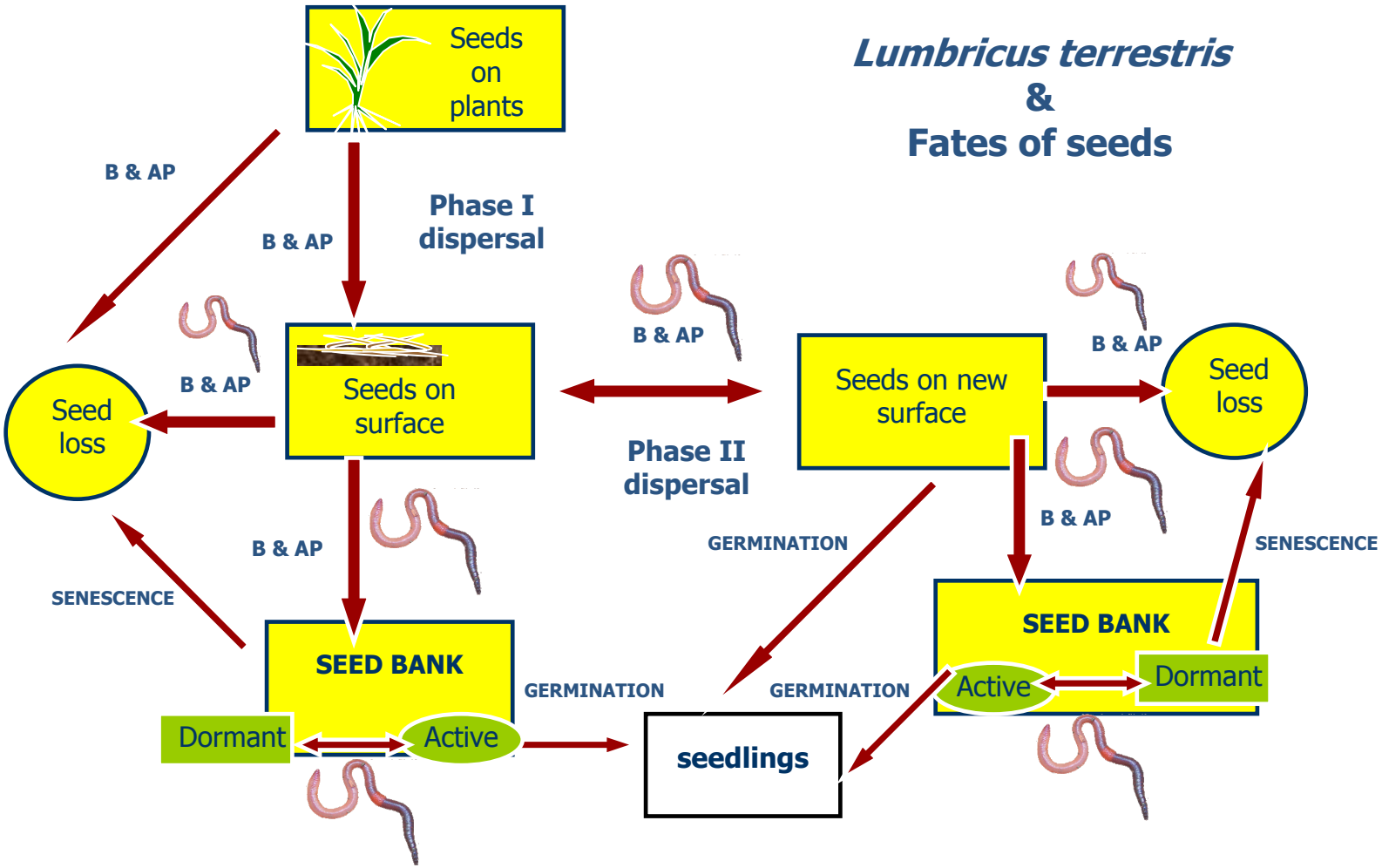

Figure 4.1 Conceptual framework of the movements and fates of seeds. Seeds can be affected by biotic processes (B) or abiotic processes (AP). As part of the biotic processes (B) earthworms point to the stages where *Lumbricus terrestris* interact with the seeds. Modified after Chambers & MacMahon (1994)

A constant number of 54 seeds per microcosm were added; in the three and six plant species mixtures the number of seeds per plant species was 18 and 9, respectively. The seeds were purchased from C. Apples Wilde Samen GmbH, Darmstadt, Germany. *Vicia cracca* was mechanically scarificated to stimulate germination.

Table 4.1 Set up of the experiment varying plant species number (1, 3, 6), functional group number (1, 3), identity (grasses (G), herbs (H), legumes (L)), and seed size (small and large) in a factorial design with and without *Lumbricus terrestris* (5 replicates each).
G: *Poa pratensis*, *Festuca pratensis*
H: *Bellis perennis*, *Tragopogon pratensis*
L: *Trifolium repens*, *Vicia cracca*

Mixture	Diversity	Species composition	FG	Seed size (mm) [a]
1	1	*P. pratensis*	G	small (1.9 x 1)
2	1	*B. perennis*	H	small (1.2 x 0.2)
3	1	*T. repens*	L	small (1.2 x 1.2)
4	1	*F. pratensis*	G	large (6 x 1.3)
5	1	*T. pratensis*	H	large (12 x 1.3)
6	1	*V. cracca*	L	large (2.8 x 2.8)
7	3	*P. pratensis, B. perennis, T. repens*	GHL	small
8	3	*F. pratensis, T. pratensis, V. cracca*	GHL	large
9	6	*P. pratensis, B. perennis, T. repens, F. pratensis, T. pratensis, V. cracca*	GHL	small + large

[a] Seed size estimates were directly measured.

Sampling and analytical procedure

During the first three weeks of the experiment the number of germinated seeds was counted twice per week in order to detect treatment effects on seed germination. The experiment lasted 7 weeks, and at the end of the experiment the established plants were counted, harvested separately and dried at 60ºC for three days

The amount of weeds germinating during the experiment was low (0.6 ± 0.8 ind./microcosm) and all germinated weeds were counted and picked. At harvest the position of each individual plant in the microcosm was recorded on a transparent map to assess the spatial pattern of the established seedlings. Scion Image program (Scion Corporation; http://www.scioncorp.com/) was used to read the Cartesian coordinates of each plant individual from the transparent maps. Earthworms were collected by hand sorting, washed, dried for 1 min on filter paper and weighed.

Analysis of variance (ANOVA) as part of the GLM procedure in SAS 8 (SAS Inst., Cary, Florida, USA) was used to analyse in a hierarchical order (sum of squares type I; SS1) the effects of *L. terrestris* (Ew), seed size (Ss), functional group diversity (FGdiv), functional group identity (grasses (G), herbs (H) and legumes (L) and interactions) on arcsine-transformed percentages of the total established plant individuals per microcosm in the six monocultures and in the three species mixtures. Additionally, in another ANOVA (sum of squares type III; SS3) we investigated how *L. terrestris* presence (Ew) and plant diversity (Div) affected the recruitment of seedlings of different species (as arcsine-transformed percentage of seedlings established per species) in the monocultures, three and six plant diversity mixtures. For the paired monocultures (with and without *L. terrestris*) repeated measures ANOVA was used to test for time effects on seed germination. The mixtures with three and six plant species were analysed by individual MANOVAs (sum of squares type III; SS3) to evaluate the effect of *L. terrestris* on plant species dominance. Plant coordinates were used to analyse effects of *L. terrestris* on the spatial distribution of individual plant species. For this the circular microcosm area was divided in 18 sections by three concentrical rings each divided in six sectors. To assess the association between earthworm burrows and the established seedlings the co-occurrence of plants und burrows (the number of sections where both, earthworm burrows and plants were present) in the 18 sections of each microcosm, averaged over identical replicates was then compared with the frequency distribution of average co-occurrences resulting from 999 randomised Monte Carlo simulations where the

observed number of plants and burrows was randomly and independently re-allocated within each pot. In addition, an index of aggregation was calculated per microcosm to characterise the degree of spatial aggregation within microcosms. The variance/mean ratio of observed numbers of plants in each subarea was not directly applicable as an index of dispersion because the 18 sections were of different size. For each subarea we therefore multiplied the number of observed plant individuals with the ratio between average and individual subarea size and used the variance/mean ratio of these area-corrected values (plants /11.17 cm^2) as an index of aggregation (note that the expected value of this index under a random pattern depends on the size distribution of the subareas and equals 1.533 in our case). The effect of *L. terrestris* and seed size on the index of aggregation was then assessed using the Mann-Whitney U-test.

4.4 Results

In less then 48 hours 95% of the added seeds were buried into earthworm burrows irrespective of seed size, plant functional group and plant species. Irrespective of plant functional group and plant diversity *L. terrestris* strongly reduced the percentage of seedlings recruited from 47 to 16% (Table 4.2). Overall, seed size affected significantly the total number of seedlings recruited per microcosm, with large size seeds having a higher recruitment rate (40%) compared to small size seeds (23%). Recruitment of plant species with small seeds was reduced stronger (-23%) in the presence of *L. terrestris* than that of plants with large seeds (-10%) (significant Ew x Ss interaction; Table 4.2, Fig. 4.1). However, some species were more affected than others; the reduction was high in *P. pratensis* (from 24 to 5%), *B. perennis* (from 56 to 6%), *T. repens* (from 43 to 10%) and *F. pratensis* (from 64 to 22%), but low in *T. pratensis* (from 54 to 38%) and *V. cracca* (from 47 to 37%; Table 4.3). In contrast, seedling recruitment in presence of earthworms generally was reduced but if legumes were present it was increased (significant Ew x L interaction; Table 4.2). Recruitment of seedlings did not vary with plant functional group diversity and identity but the recruitment and individual biomass of the grass species (*P. pratensis* and *F. pratensis*) were affected by the earthworm presence and plant diversity (Ew x Div). In addition, there was a strong interaction between *L. terrestris*, seed size and functional group identity (grasses, herbs or legumes); recruitment of grass species with small seeds was generally low (Ew x Ss x G), recruitment of herb species was high in treatments without *L. terrestris* irrespective of seed size (Ew x Ss x H), whereas

recruitment of legume species with large seeds was high in presence of *L. terrestris* (Ew x Ss x L) (Fig. 4.2, Table 4.3).

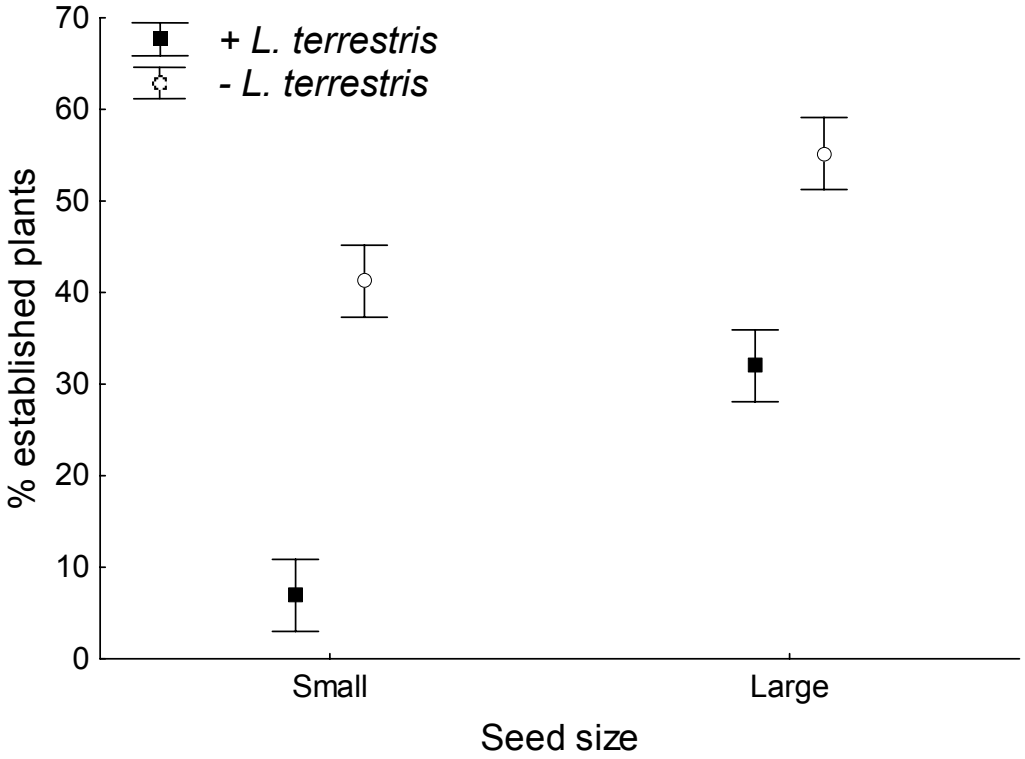

Figure 4.1 Percentage of recruitment of plant species as affected by seed size and *Lumbricus terrestris*. Error bars represent ± SE.

In contrast to seedling recruitment, *L. terrestris* significantly increased the aboveground biomass of individual plants at the end of the experiment in *Poa pratensis* (+138% $F_{1,18}$ = 12.87, P =0.005), *F. pratensis* (+223%; $F_{1,18}$ = 18.4, P < 0.001), *T. pratensis* (+124%; $F_{1,18}$ = 17.1, P = 0.005), *V. cracca* (+48%; $F_{1,18}$ = 5.9, P = 0.023) and in tendency also in other species (Fig. 4.3, Table 4.3).

V. cracca was the only species where *L. terrestris* accelerated germination with significantly more individuals germinating in the first week than in the control (+128%; $F_{3,6}$ = 4.8, P = 0.048). However, after 3 weeks the number of recruited seedlings in the control equalled that in the earthworm treatment and at the end of the experiment there were fewer plants in presence of earthworms.

Table 4.2 ANOVA (SS1) table of F-values on the effect of *L. terrestris* (Ew), seed size (Ss), functional group diversity (FGdiv), identity of the functional groups (grasses (G), herbs (H), legumes (L)) on the arcsin-transformed percentage of total recruited seedlings in the monocultures and three species mixtures.

	F-values
Ew	**409.5*** **
Ss	**113.4*** **
Ew x Ss	**19.1*** **
FGdiv	0.5
G	1.0
H	0.7
L	0.2
Ew x G	0.3
Ew x H	0.11
Ew x L	**24.9*** **
Ss x G	3.8^{+}
Ss x H	2.4
Ss x L	0.1
Ew x Ss x G	**24.3*** **
Ew x Ss x H	**11.7** **
Ew x Ss x L	**18.2*** **

***, $P < 0.001$; **, $P < 0.01$; $^{+}$, $P < 0.10$

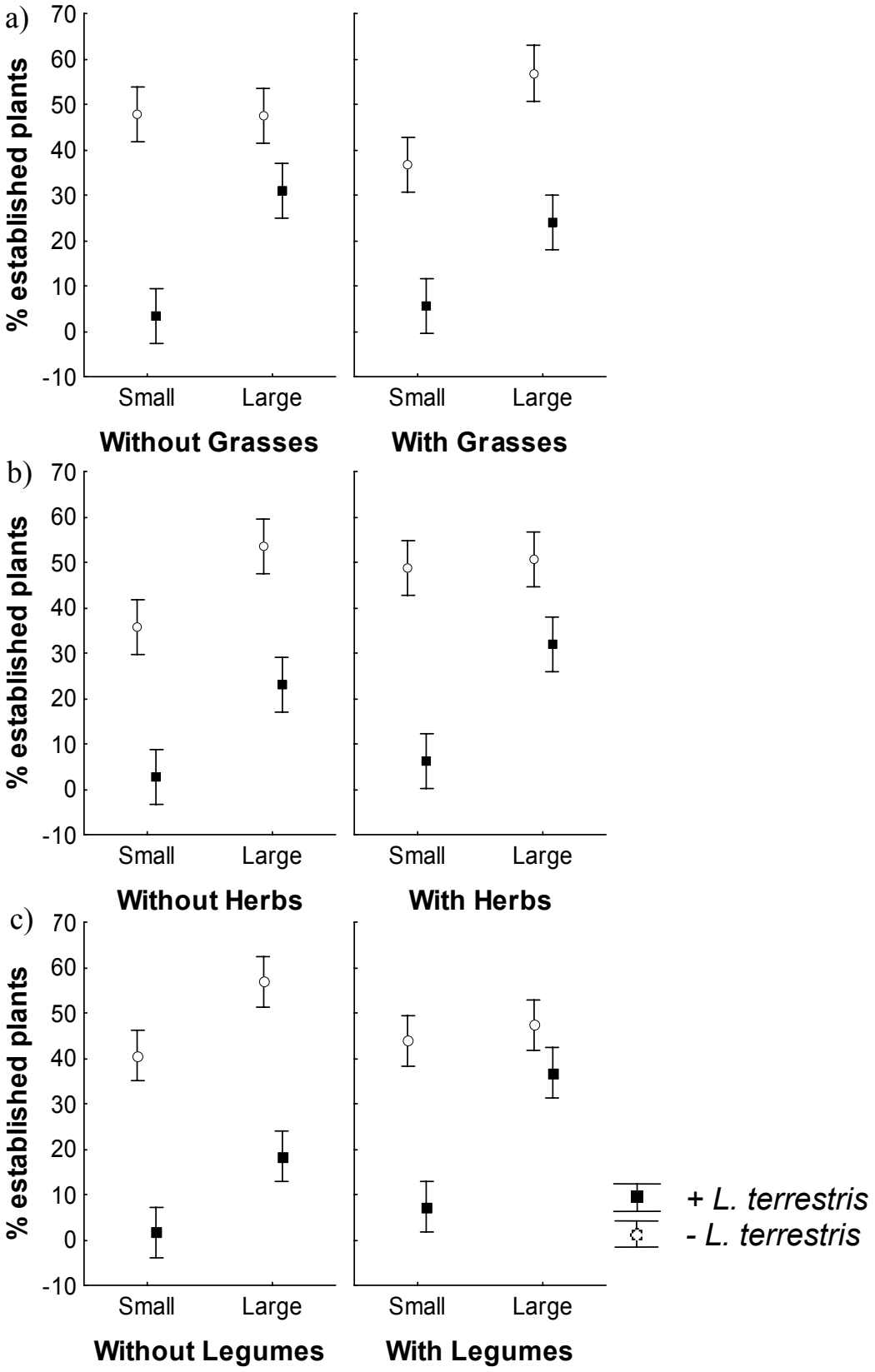

Figure 4.2 Percentage of recruitment of plants differing in seed size (small and large) as affected by *Lumbricus terrestris* and the presence of (a) grasses, (b) legumes and (c) herbs. Error bars represent ± SE.

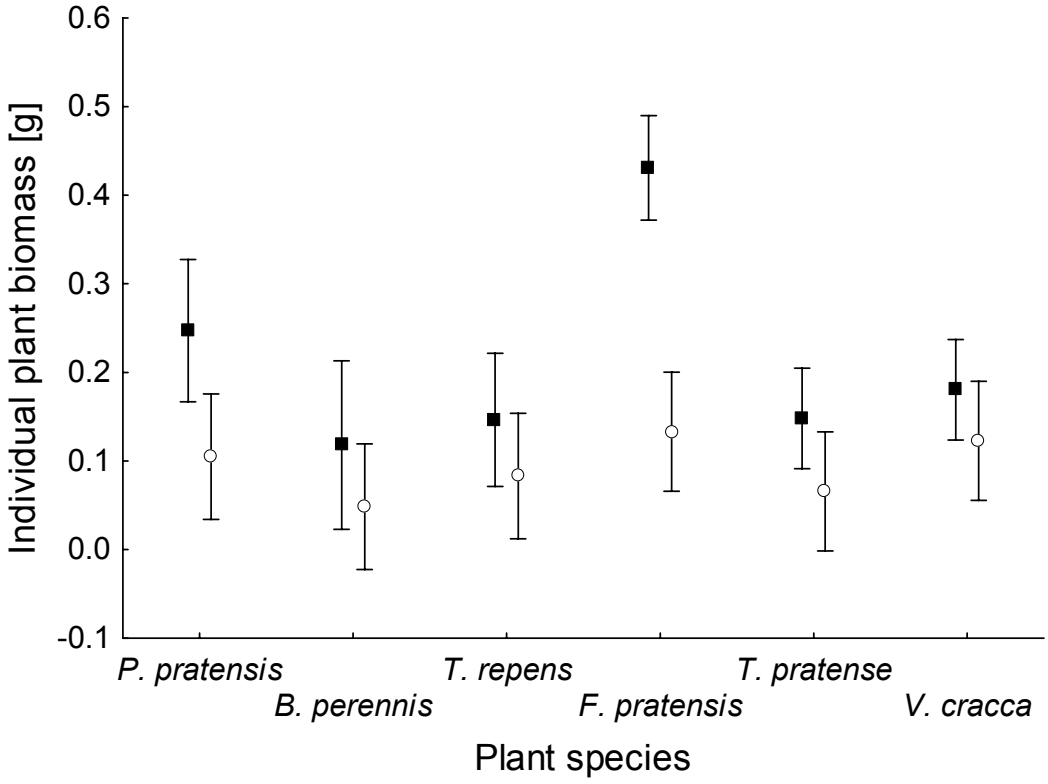

Fig. 4.3 Mean biomass (dry weight) of plant individuals as affected by *L. terrestris* presence. Error bars represent ± SE. For full species names see Table 4.1.

In the three species mixtures *L. terrestris* significantly affected the relative abundances of plant species within communities with small (Pillai's Trace, $F_{3,6}$= 49.87, P < 0.001) and large seeds (Pillai's Trace, $F_{3,6}$= 43.19, P < 0.001) and this was also true for the six species mixture (Pillai's Trace, $F_{6,2}$= 42.58, P = 0.023) (Fig. 4.4). Standardised canonical coefficients suggest that *B. perennis* contributed most to the change in the composition of plant species in small and in all six species seed mixtures, while in the large seed mixtures *F. pratensis* was the species which contributed most (Table 4.4). Furthermore, significantly more weed seedlings established in the presence of *L. terrestris* (0.91 seedlings per microcosm) than in the control (0.28 seedlings per microcosm; $F_{1,88}$ = 14.5, P < 0.001).

Table 4.3 ANOVA (SS3) table of F-values on the effect of *Lumbricus terrestris* (Ew), plant species diversity (Div) and the interaction (Ew x Div) on the recruitment of seedlings of different species (as arcsin-transformed percentages of the number of seeds added per species) and on the biomass of plant individuals at the end of the experiment from monocultures, three and six species mixtures.

Seed size	**Small**						**Large**					
Plant species	*P. pratensis*		*B. perennis*		*T. repens*		*F. pratensis*		*T. pratensis*		*V. cracca*	
	recruitment	biomass	recruitment	biomass	recruitment	biomass	recruitment	biomass	recruitment	biomass	recruitment	biomass
Ew	**29.8** ***	**12.8** **	**209.5** ***	3.4 NS	**89.5** ***	0.4 NS	**114.4** ***	**18.4** **	**11.2***	**17.1****	**12.1***	3.9 NS
Div	0.3 NS	**18.5*****	1 NS	1.5 NS	1.0 NS	1.6 NS	1.6 NS	0.6	1.4 NS	2.3 NS	0.7 NS	0.6 NS
Ew x Div	0.9 NS	**19.0*****	1 NS	0.9 NS	0.3 NS	0.3 NS	**4.3***	**4***	0.8 NS	0.8 NS	2.5 NS	0.1 NS

*****, P < 0.001; **, P < 0.01; *, P < 0.05**

Aggregation of plants was significantly increased by *L. terrestris* ($Z = 6.71$, $P < 0.001$) (Fig. 4.5); with plants being associated with earthworm burrows except for the legumes *T. repens* and *V. cracca* (Table 4.5). Seed size had no significant effects on spatial aggregation ($Z = 0.67$, $P = 0.505$).

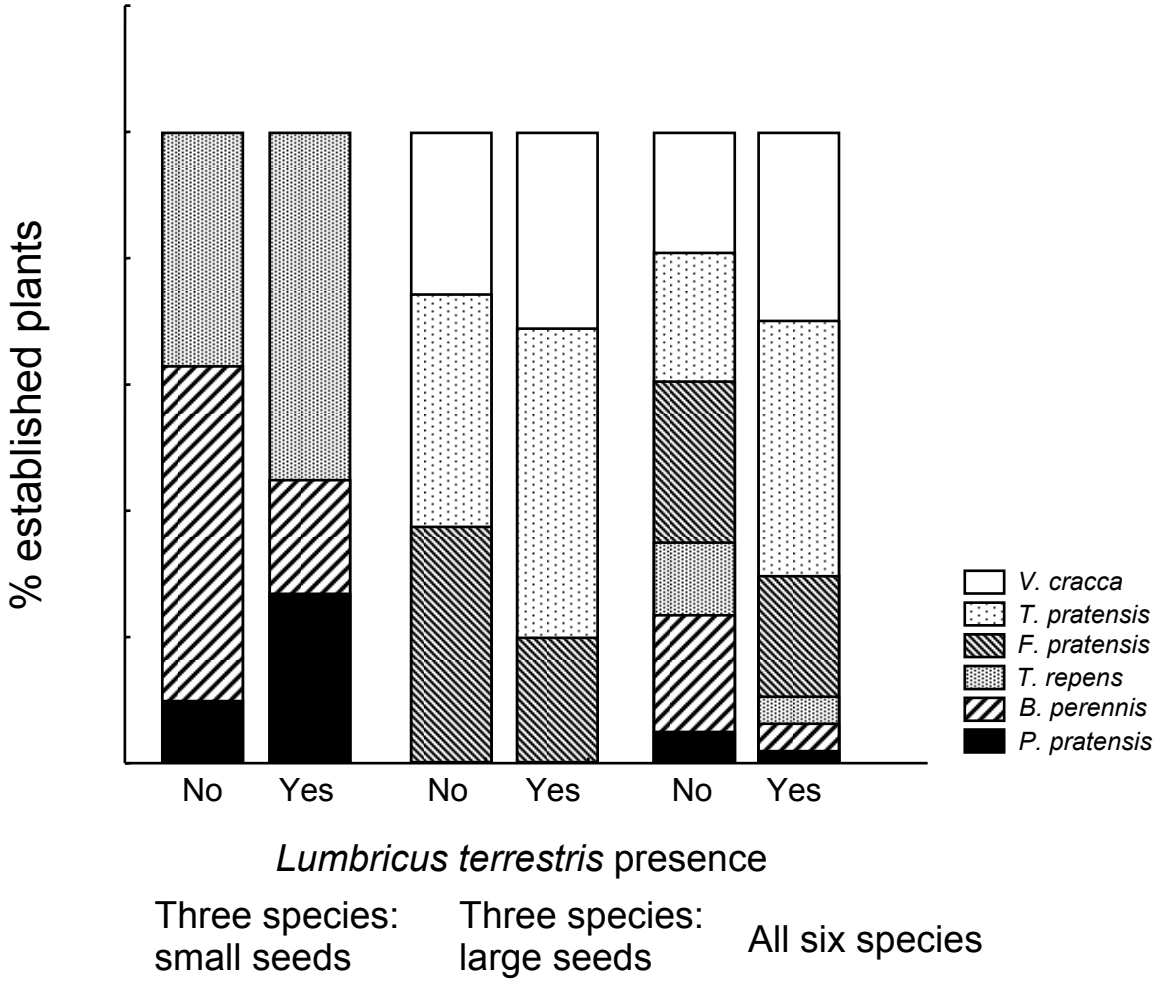

Figure 4.4 Changes in plant species composition (percentage of established) as affected by *Lumbricus terrestris* presence and seed size. For full species names see Table 4.1.

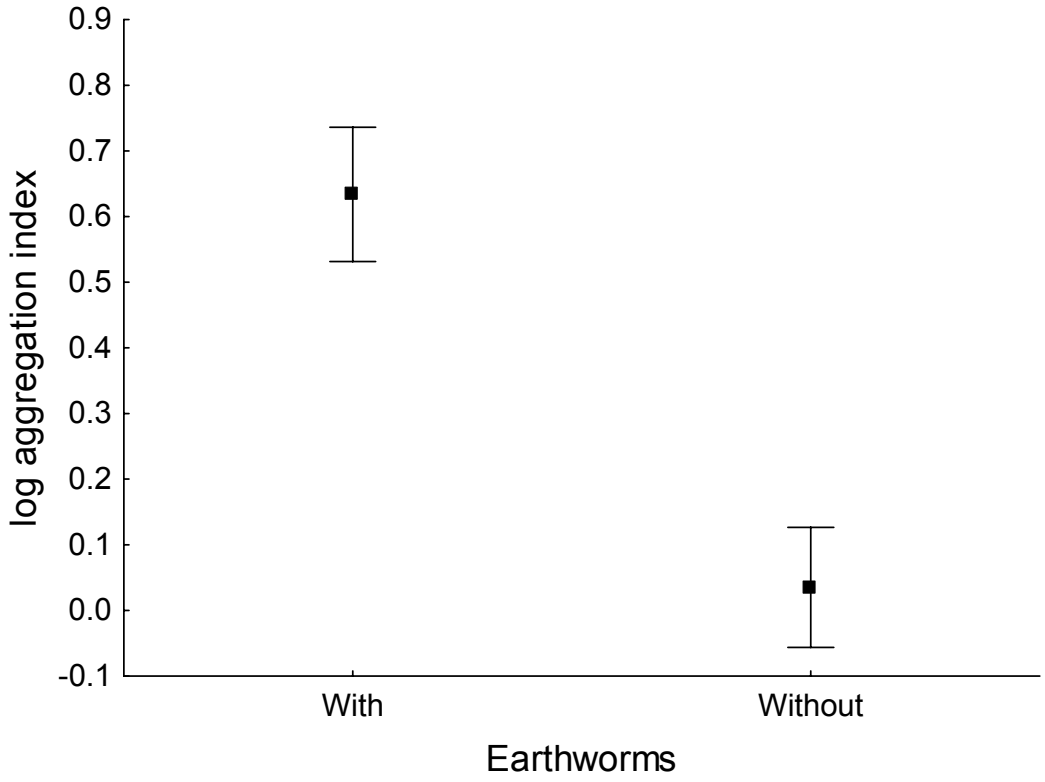

Fig. 4.5 Index of aggregation (log-transformed) for recruited plant seedlings in earthworm/no earthworm treatments. Error bars represent ± SE.

Table 4.4 Standardized canonical coefficients reflecting the contribution of individual plant species to changes in plant community composition in the mixtures with three plant species (small and large) and in the mixtures with all six plant species as affected by *Lumbricus terrestris.*

	Three plant species with small seeds	Three plant species with large seeds	All six plant species
	Standardised canonical coefficients		
P. pratensis	-0.12	-	1.42
B. perennis	**5.08**	-	**6.47**
T. repens	-0.23	-	0.58
F. pratensis	-	**4.53**	1.68
T. pratensis	-	-0.47	-1.80
V. cracca	-	0.76	3.65

Table 4.5 Association between established plant seedling and burrows. The average number of co-occurrence of plants and burrows in the microcosms with *L. terrestris* is used as a test statistic in a Monte Carlo test to compare with the frequency distribution of simulated co-occurrences resulting from 999 Monte Carlo simulations where the observed number of plants and burrows was randomly and independently re-allocated within each pot.

Mixture	Diversity	FG	Seed size	Average expected co-occurrence	Average observed co-occurrence	P-value
1	*P. pratensis*	G	Small	0.19	0.40	**0.032**
2	*B. perennis*	H	Small	0.21	0.60	**0.003**
3	*T. repens*	L	Small	0.71	0.80	0.235
4	*F. pratensis*	G	Large	1.11	2.80	**0.001**
5	*T. pratensis*	H	Large	2.17	3.60	**0.002**
6	*V. cracca*	L	Large	3.37	3.6	0.245
7	*P. pratensis, B. perennis, T. repens*	GHL	Small	0.36	1	**0.002**
8	*F. pratensis, T. pratensis, V. cracca*	GHL	Small	3.21	3.8	0.068
9	*P. pratensis, B. perennis, T. repens, F. pratensis, T. pratensis, V. cracca*	GHL	Small + Large	1.64	2.8	**0.002**

4.5 Discussion

In the present experiment plant seeds were buried quickly by *L. terrestris*, within 48 h, regardless of size, species or functional group. In previous studies *L. terrestris* selectively fed on plant seeds of a certain size, shape and surface texture (Mc Rill, 1974; Grant 1983); selective feeding on leaf litter by *L. terrestris* also varies with these parameters (Satchel and Lowe 1967). We did not distinguish between seeds which were ingested and those which were only pulled into the burrow and buried. Since *L. terrestris* is unable to feed on particles with a diameter larger than 2 mm (Shumway & Koide 1994, Schulmann & Tiunov 1999) some of the seeds, such as those of *V. cracca* and *T. pratensis,* certainly were too large for being swallowed.

Germination rates were generally reduced in presence of *L. terrestris* irrespective of plant species and seed size. However, plant species with small seeds were more strongly affected, presumably due to digestion or damage during the passage through the gut of *L. terrestris* (McRill & Sagar 1973, Shumway & Koide 1994), or due to burial below the germination level. On the other side, the big size seeds were not ingested, but have been buried mostly in the upper 3-4 cm of the soil or were used together with litter to build up middens on the burrow entry, which are typical for anecic earthworms. Since seeds at the soil surface are more vulnerable to predation (Harper 1957, Chambers & MacMahon 1994, Wilby & Brown 2001) and germination might be hampered by the lack of water, seeds may also benefit from being buried by earthworms. In particular, seeds surviving the gut passage may find favorable environmental conditions for germination, seedling establishment and growth due to increased water holding capacity and concentrations of nutrients in earthworm casts and middens, in particular N and P (James 1991, Blanchart et al. 1999). Supporting this view the aboveground biomass of individual plants growing in earthworm treatments was significantly increased in *P. pratensis, F. pratensis* and *T. pratensis* and in tendency also in the other species. Shoot biomass increased probably also in part due to the reduced intra- and interspecific competition since the number of established plants was lower in the microcosm with *L. terrestris*. Higher water content in earthworm casts presumably beneficially affected germination of seeds of *V. cracca* since seeds of this species are known to germinate only at high humidity. In the six species mixture plant species with large seeds dominated in presence of *L. terrestris* whereas *B. perennis*, a species with small seeds, was detrimentally affected.

The fast removal of the seeds (especially of the large seeds that cannot be ingested) from the surface independent of size, surface texture or plant functional group suggests that studies using seed removal to quantify seed predation may be misleading in grasslands colonized by *L. terrestris* (Vander Wall, Kuhn & Beck 2005). The strong interactions between *L. terrestris*, seed size, plant functional group identity (grasses, herbs, legumes) and seedling establishment, i.e. the differential reduction/promotion of germination of seeds of different size and plant functional group by *L. terrestris* suggests that anecic earthworm species strongly affect plant recruitment and ultimately also the composition of plant communities.

The significant association of recruiting plants with earthworm burrows in the present study is consistent with Grant's (1983) finding that 70% of the seedlings in temperate grasslands germinate out of earthworm casts, although they covered only 24-28% of the soil surface. The strong aggregation of plants in the vicinity of earthworm burrows resulted in plant communities with a more heterogeneous small-scale architecture, dense spots and large gaps in earthworm treatments as compared to the control with a more uniform plant distribution. Therefore, the increase in microhabitat heterogeneity and the small scale disturbance caused by anecic earthworms, such as *L. terrestris*, may generally promote plant diversity as suggested by the intermediate disturbance hypothesis (Connell 1978, Fox 1979). A number of authors including Pickett (1980), Pickett and White (1985), Shmida & Ellner (1984) and Wilson (1994) have pointed out that the intermediate disturbance hypothesis of species coexistence is based on patch dynamics.

Weeds germinating in the microcosms during a period of four weeks before the start of the experiment were removed. Nevertheless, at the end of the experiment the number of weeds in the microcosms with *L. terrestris* were significantly increased suggesting that the earthworms translocated seeds from deeper in the soil to the soil surface where they germinated. Again, this is consistent with the finding of Grant (1983) that plant recruitment of plant seedlings in grasslands predominantly occurs in earthworm casts. In natural grasslands the area in between earthworm burrows is also colonized by plants and in these areas seedling recruitment likely is hampered due to competition. Therefore, the increase in spatial heterogeneity and disturbance by anecic earthworms, i.e. the deposition of casts and the formation of patches with bare soil, likely contributes to reduce competitive exclusion and therefore to maintain high plant species richness in grasslands.

Indeed, Kotorova & Leps (1999), Jakobsson & Eriksson (2000) and Moles & Westoby (2004) showed that the recruitment of seedlings in natural grasslands is species specific, but generally beneficially affected by disturbance with large seeds having the highest recruitment rates. In addition, burial of seeds by anecic and also other earthworms significantly contributes to the build up of the soil seed bank (Grant 1983 van der Reest & Rogaar 1988, Willems & Huijsmans 1994). Since the soil seed bank strongly contributes to the resilience of grassland communities speeding up regeneration following disturbances (Uhl et al. 1981, Marks & Mohler 1985, Kalamees & Zobel 2002) earthworms play an important role in the stability of grassland systems. Improving seed germination and seedling recruitment by earthworms likely more than compensates for seed digestion during the gut passage through earthworms which is known to be low (McRill & Sagar 1973, Grant 1983). Therefore, earthworms likely form an indispensable component of terrestrial ecosystems contributing to the maintenance of plant species diversity and plant community stability, in particular in grassland ecosystems. However, detrimental effects of earthworms on herbaceous plants and tree seedlings also have been documented (James & Cunningam 1989, Gundale 2002, Hale 2004), but these reports are restricted to habitats previously devoid of earthworms and now invaded by non-native earthworm species (Bohlen et al. 2004, Hale 2004).

In conclusion, our findings suggest that secondary seed dispersal by anecic earthworm species, such as *L. terrestris* and probably also of other earthworm species, forms a major driving force for seed germination, seedling recruitment, plant community composition and the stability of grassland communities. Therefore, the role of earthworms in the formation of plant communities and plant succession deserves further attention. Fast removal of seeds from the soil surface, selective promotion or repression of seedling germination, increasing microhabitat heterogeneity and seed bank formation are prominent mechanisms by which earthworms affect seed germination, seedling recruitment, plant community composition and maintenance of plant diversity.

Chapter 5

Earthworms, microorganisms and litter decomposition as affected by plant species and plant functional group diversity

5.1 Abstract

Litter decomposition as affected by earthworms, springtails, microorganisms and increasing plant species diversity (1, 4, 16) and number of functional groups (grasses, herbs, legumes) was studied in an experimental grassland ("The Jena Experiment", Jena, Germany). Decomposer treatments with increased and reduced earthworms density, natural and reduced springtail populations and control were established in each plant species diversity mixtures. Litter bags containing litter of three plant functional groups (grasses, herbs legumes) separately and as a mixture were placed in experimental plots.

Increasing plant species and functional group diversity lead to an increase in earthworm biomass and density as well as an increase in microbial respiration, but without any effects on earthworm species composition. Legume presence and soil properties affected the species composition of the earthworm community. Presence of legumes was responsible for increasing earthworm density and biomass with increasing plant species diversity, but not for the increase in microbial respiration.

Litter decomposition was not significantly affected by plant species diversity. Increasing functional group diversity increased the decomposition of legume litter. Increased earthworm density also accelerated litter decomposition. Decomposition of legume litter was fastest in the treatment with increased density of earthworms.

Strong block effects on decomposition suggest that environmental factors (soil silt content, water holding capacity) were the main driving factors of the decomposition process and that plant species and functional group diversity played a minor role. The results also indicate that organic matter rich in nitrogen decomposed faster and that litter feeding and burrowing macroinvertebrates like earthworms play an important role. Furthermore, the increase in earthworm densities with increasing species and functional group diversity suggest a higher nitrogen turnover with increasing diversity.

5.2 Introduction

The current rate of biodiversity loss greatly exceeds the rate that nature can compensate for and adapt to. Today species become extinct 100-1,000 times faster than before human times and for every 10,000 species that go extinct, only one new species evolves (Chapin et al. 1998). Biodiversity significantly affects ecosystem functioning, but the relative importance of the mechanisms involved in this interrelationship are still disputed (Kaiser 2000, McCann 2000, Wardle et al. 2000).

Despite consensus has been reached that species complementarity contributes to the relationships between biodiversity and ecosystem functioning, still there are a number of unresolved questions (Hooper et al. 2005). An important unresolved question is to what extent decomposition and decomposer performances are affected by plant diversity. Some authors found positive relationships (Zaller & Arnone 1999, Stephan et al. 2000, Spehn et al. 2000), others no consistent or idiosyncratic responses (Wardle et al. 1997, Wardle et al. 1999, Knops et al. 2001, Gastine et al. 2003, Hedlund et al. 2003, Salamon et al. 2004).

Decomposers are a critical component of terrestrial ecosystems because they regulate decomposition and nutrient mineralization and therefore ultimately the aboveground producers (Swift et al. 1979, Schlesinger 1997). Plants on the other side affect the decomposer subsystem via the quality and quantity of the litter produced (Wardle et al. 1995, Groffman el al. 1996), but also via the amount and compostion of root exudates (Li et al. 2004, Bais et al. 2004), and via competition for nutrients (Grime 1994, Fransen et al. 1999).

Manipulating in one experiment both compartments, the aboveground (plant diversity) and belowground (decomposer densities) allow to investigate relationships between decomposition and plant diversity. In experimental grassland communities with varying plant species diversity (1, 2, 4, 8, 16, and 60) and plant functional group diversity (1, 2, 3, and 4) (Roscher et al. 2004) we investigated the decomposition of litter as affected by plant species and functional group diversity.

Belowground we focused on two decomposer groups, earthworms (Lumbricidae) and springtails (Collembola). Earthworms because they are major ecosystem engineers in grasslands and key decomposers, being able to affect plant performance (Scheu et al. 1999, Wurst et al. 2003), soil physical properties (Lee & Foster 1991, Lavelle et al. 1997), alter soil microbial community composition and functioning (Brown 1995, Scheu 2002), increase

nutrient cycling (Edwards & Bohlen 1996, Parmelee et al. 1989) and affect plant growth and vegetation development (Schmidt & Curry 1999, Zaller & Arnone 1999, Thompson et al. 1993, Scheu 2003). Springtails affect plant nutrient availability by grazing on microorganisms, thereby affecting the structure and functioning of the microbial community in the rhizosphere (Rusek 1998, Gange 2000).

Concomitant with varying plant species and functional group diversity two earthworm treatments, increased and reduced earthworm density were created. Springtail populations were also manipulated in two treatments, with natural and reduced springtail populations.

These treatments were used to investigate the decomposition of litter as affected by plant species and functional group diversity and earthworm and springtail density. Aditionally, since soil processes are essentially controlled by soil microorganisms (Balser & Firestone 2002) we also investigated effects of plant diversity and decomposers on microbial biomass and activity.

We hypothesised (1) that increasing plant species and functional group diversity will have positive effects on earthworm and springtail densities, litter decomposition and microbial activity and biomass, (2) that manipulations in decomposer densities (earthworms and springtails) will affect microbial community and litter decomposition and that the effect of the animals will vary with litter functional group and diversity, and (3) that these effects depend on plant species diversity, functional group diversity and functional group identity of the plants.

5.3 Materials and methods

"The Jena Experiment" design

The study was carried out near the Saale river (altitude 130 m NN, Turingia, Germany). The study system was a typical Central European mesophilic grassland traditionally used for haymaking (Ellenberg 1988). Mean annual air temperature 3 km south of the field site is 9.3 °C and annual precipitation is 587 mm. A pool of 60 native plant species was used to establish by independent random draws with replacement a gradient of plant species (1, 2, 4, 8, 16, 60) and functional group diversity (1, 2, 3, 4) in a total of 90 plots of 20 x 20 m (Appendix 1, 2). There were 16 replicates for mixtures with 1, 2, 4, 8, species diversity and 14 replicates for mixtures with 16 species, and 4 replicates for the mixtures with 60 species.

Additionally, 4 plots with bare ground, 2 plots with free succession and 2 plots with succession with mowing were established (Roscher et al. 2004). The plots were weeded two times per year to maintain the target species.

Plant functional groups were assessed using three classes of attributes: (1) above- and below-ground morphological traits (2) phenological traits and (3) the ability for N_2 fixation. Seventeen variables of the selected species attributes were analysed by a multivariate cluster method (Ward's method, Euclidian distance, Kaufman & Rousseeuw 1990) in order to identify species functional groups (Roscher et al. 2004). In each plot three randomly selected subplots of 2 x 4 m were used to create the following decomposer treatments: control, earthworms (increased and reduced) and springtails (reduction).

Manipulation of earthworm densities

The 2 x 4 m earthworm subplot was further divided into two earthworm density treatments. The subplots were 1 x 1 m and were enclosed with PVC shields, aboveground (20 cm) and belowground (15 cm), with 50 cm distance between them. The above ground shields were sufficient to prevent the escape of *L. terrestris,* while those belowground presumably prohibited exchange of endogeic earthworm species but less than of the anecic *L. terrestris*. The subplots were built only in the 1, 4, 16 plant species diversity levels.

In the increased earthworm density treatment 25 adults of *L. terrestris* (average fresh weight 4.2 ± 0.94 g) were added in each enclosure per year. The addition of *L. terrestris* started in September 2003 and continued in 2004 and 2005. The aboveground part of the shields were shortly removed during mowing periods (14 days, two times per year).

Oktett method (Tieleman 1989) was used to extract the earthworms in the enclosures with reduced earthworm density. Four devices (DEKA 4000, Deka Gerätebau, Marsberg, Germany) were simultaneously used to cover the 1 m^2 area of the plots, and the extraction was performed two times per year, in spring and autumn. Two 75 Ah car batteries were simultaneously used to supply electricity. In each enclosure the earthworm extraction was performed for 35 minutes increasing the voltage form 250 V (10 min) to 300 V (5 min), 400 V (5 min), 500 V (5 min) and 600 V (10 min).

Manipulation of springtails density

Reducing the density of springtails was done by spraying a contact insecticide one time per month between April and October (Hortex (chlorpyrifos 2% w/w) 40 g in 1 l water, 1 l per plot; Celaflor, Dow Agrosciences LCC, USA). The insecticide was found to strongly reduce springtail density (Brown & Gange 1989) without affecting earthworms (Brown & Gange 1989b, Domsch 1992).

Litter bag experiment

Litter of three plant functional groups (grasses, herbs and legumes) were used to establish four litter treatments (grasses (Gl), herbs (Hl), legumes (Ll) and mixed Ml). Each litter bag contained 3 g dry weight of plant material. Each plant functional group was obtained by mixing 1 g of three plant species: grasses (*Festuca rubra, Lolium perene, Poa pratensis*) (N 2.0%, C:N 22.6), herbs (*Cirsium oleraceum, Daucus carota, Plantago lanceolata*) (N 2.3%, C:N 19.6), legumes (*Lathyrus pratensis, Lotus corniculatus, Trifolium repens*) (3.0%, C:N 15.5). For the mixed litter treatment 3 g dry weight from a homogenous mixture created by mixing all 9 plant species (N 2.4%, C:N 22.3) was used. The litter material was collected form the Jena Experiment field site in the previous season (2003), sorted, dried 3 days at 60°C and cut into pieces about 3 cm in length. A 4 mm mesh was used in order to allow access of macrofauna (earthworms).

Analyses

Earthworms extracted from the reduced density plots were identified to species level, counted and weighed.

Litterbags of each of the four litter treatments were placed on the soil surface of the control, increased earthworm density and springtail reduced density treatments, of the 1, 4, 16 plant species diversity plots, as well as in bare ground free succession and succession with mowing plots (February 2004). The litter bags were collected in June, dried 3 days at 60 C° and weighed.

Samples for determination of the microbial respiration and biomass were taken one year after the establishment of the plant communities (April 2003), to analyse early plant species or functional group diversity effects. In September 2004 we performed a second analysis and included our decomposer treatments. The soil (pH = 7.1 – 8.4, C_{org} = 5-33 g Kg^{-1}, N_{tot} = 1-2.7

g N Kg^{-1}) from three 5 cm cores from each treatment were sieved (1 mm) and homogenised. Microbial biomass was measured using the substrate-induced respiration (SIR) method (Anderson & Domsch 1978). The microbial respiratory response to addition of glucose was measured at hourly intervals in an electrolytic O_2 microcompensation apparatus for 24 hours at 22°C (Scheu 1992). Microbial biomass (C_{mic}; µg C g^{-1}soil) was measured after the addition of a sufficient amount of glucose as substrate in order to saturate the catabolic activity of microorganisms (4 mg glucose g^{-1} soil dry weight). The maximum initial respiratory response (MIRR; µg O_2 g^{-1} soil dry weight h^{-1}) was calculated as the average of the lowest three readings within the first 11 h and microbial biomass was calculated as C_{mic} = 38 x MIRR (µg C_{mic} g^{-1} soil dry weight) (Beck et al. 1997). Soil basal respiration (µl O_2 g^{-1} soil dry weight h^{-1}) was measured as mean of the O_2 consumption rates of unamended soil of hours 15 to 20 after start of the measurements.

Data analysis

The effects of block, decomposer treatments (increased earthworm densities, reduced earthworm densities, reduced springtails densities, Control), functional group diversity (FG), plant species diversity (S), and presence/absence of legumes, grasses, small herbs and tall herbs as treatment factors were analysed using general linear models in a hierarchical order (type I sum of squares) implemented in the statistical software package SAS 8 (SAS Inst., Cary, Florida, USA). In the litter decomposition experiment, litter type (grass litter, herbs litter, legumes litter, mixed litter) was included as an additional factor.

The experimental design does not allow to fully separate the effects of S and FG which are partially confounded; the F-values given in text and tables for the effects of S (log-linear and deviation) and FG (linear and deviation), and their interactions with other factors refer to those where the respective factor (and interaction) was fitted first (Neter and Wasserman 1974, Schmidt et al. 2002). No interaction term between S and FG was calculated. The effects of presence/absence of legumes (L), grasses (G), small herbs (Sh) and tall herbs (Th) and their interactions with the decomposer treatments were always fitted after fitting S and FG. For the litter decomposition data the small herbs and tall herbs functional groups were analysed together as herbs (H). In analyses of covariance (ANCOVA) functional group identity (legumes, grasses, small herbs or tall herbs) was fitted as covariable to separate effects caused by presence of a particular functional group from diversity effects; covariables

were always fitted first. Interactions between factors that were not significant were excluded from the model.

For the comparison of decomposer treatments on microbial biomass and respiration, and on litter decomposition we used the split-plot ANOVA approach.

Treatments analysed on the scale of plots (block, FG, S, L, H, Sh Th and the interactions) were tested against the residual variability between plots (plotcode). Treatments analysed on the scale of subplots (decomposer treatments were tested against the residual variability between subplots (plotcode x treatment interaction), while the treatments nested in the subplots (litter type) were tested against the residual variability within subplots. Prior to ANOVAs, data were inspected for homogeneity of variance and log-transformed if required.

5.4 Results

Earthworms

A total of five earthworm species belonging to two functional groups (Bouché 1977), anecic (*L. terrestris*) and endogeic (*Aporrectodea caliginosa, A. rosea, Allolobophora chlorotica* and *Octolasion tyrteum*) were found. On average ca. 60 g fresh weight m^{-2} of earthworms and 96 individuals m^{-2} were extracted in autumn 2004. A strong block effect was observed, with generally higher density and biomass in block 1 (108 ± 8 ind. m^{-2}, 63 ± 7 g m^{-2}) and 2 (123 ± 9 ind. m^{-2}, 70 ± 8 g m^{-2}) close to Saale river compared to block 3 (84. ± 9 ind m^{-2}, 49 ± 8 g m^{-2}) and 4 (72 ± 8 ind. m^{-2} and 53 ± 7 g m^{-2}) (Table 5.1), except for of *A. caliginosa* with low density and biomass in block 1 (28 ± 6 ind. m^{-2}, 11 ± 2 g m^{-2}) and a maximum in block 2 (50 ± 6 ind. m^{-2}, 19 ± 2 g m^{-2}).

Species composition of earthworms was not affected by plant species and functional group diversity. Block (Pillai's Trace: $F_{15,96} = 3.91$, $P < 0.001$) and presence of legumes (Pillai's Trace: $F_{5,30} = 5.64$, $P < 0.001$) affected the relative biomass of earthworm species. The block effect was mainly caused by *A. chlorotica* with its relative biomass decreasing form 9% in block 1 to about 1% in block 4. The legume effect was caused mainly by *L. terrestris* with its biomass dominance decreasing from 73% in plots with legumes to 64% in plots without legumes. The relative abundance of earthworms was only affected by block (Pillai's Trace: $F_{15,96} = 4.17$, $P < 0.001$).

Increasing plant species diversity lead to a linear increase in total earthworm density from 85 ± 9 to 108 ± 9 ind. m^{-2} (Fig. 5.1a) and biomass, from 54 ± 6 g m^{-2} in the monocultures to 66 ± 7 g m^{-2} in the 16 species mixtures (Table 5.1, Fig. 5.1b).

Table 5.1 ANOVA table of F-values on the effect of block, functional group diversity (FG), species diversity (S) and presence/absence of legumes (L), grasses (G), small herbs (Sh), tall herbs (Th) on earthworm density, earthworm biomass and *Lumbricus terrestris* biomass.

Treatment factors	**Earthworm densities**	**Earthworm biomass**	***L. terrestris* biomass**
Block	$F_{3/34} = 10.25$***	$F_{3/34} = 3.59$*	$F_{3/34} = 2.12$
FG	$F_{3/34} = 3.04$*	$F_{3/34} = 0.79$	$F_{3/34} = 0.40$
FG log linear	$F_{1/34} = 4.93$*	$F_{1/34} = 1.91$	$F_{1/34} = 0.85$
FG deviation	$F_{2/34} = 2.10$	$F_{2/34} = 0.22$	$F_{2/34} = 0.18$
S	$F_{1/34} = 4.02$*	$F_{1/34} = 2.38^{+}$	$F_{1/34} = 1.18$
S log linear	$F_{1/34} = 7.93$**	$F_{1/34} = 4.61$*	$F_{1/34} = 2.19$
S deviation	$F_{5/34} = 0.11$	$F_{1/34} = 0.15$	$F_{1/34} = 0.17$
L	$F_{1/34} = 9.26$**	$F_{1/34} = 17.94$**	$F_{1/34} = 13.60$**
G	$F_{1/34} = 5.4$*	$F_{1/34} = 9.19$ **	$F_{1/34} = 7.15$*
Sh	$F_{1/34} = 2.14$	$F_{1/34} = 0.0$	$F_{1/34} = 0.11$
Th	$F_{2/34} = 4.71$*	$F_{2/34} = 1.61$	$F_{2/34} = 0.45$

*****, P < 0.001; **, P < 0.01; *, P< 0.05**

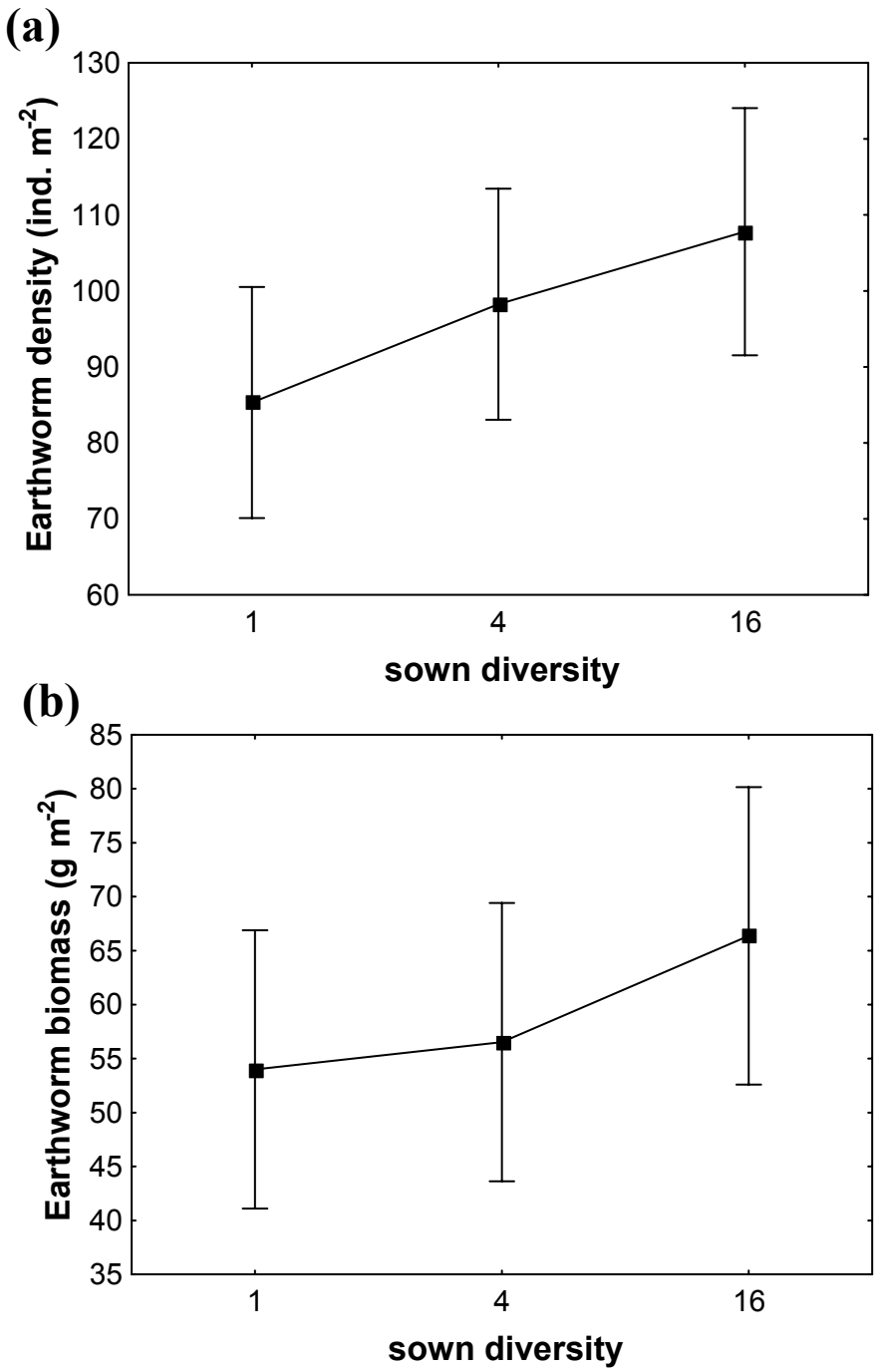

Figure 5.1 (a) Earthworms density and (b) biomass as affected by plant species diversity. Error bars represent ± SE.

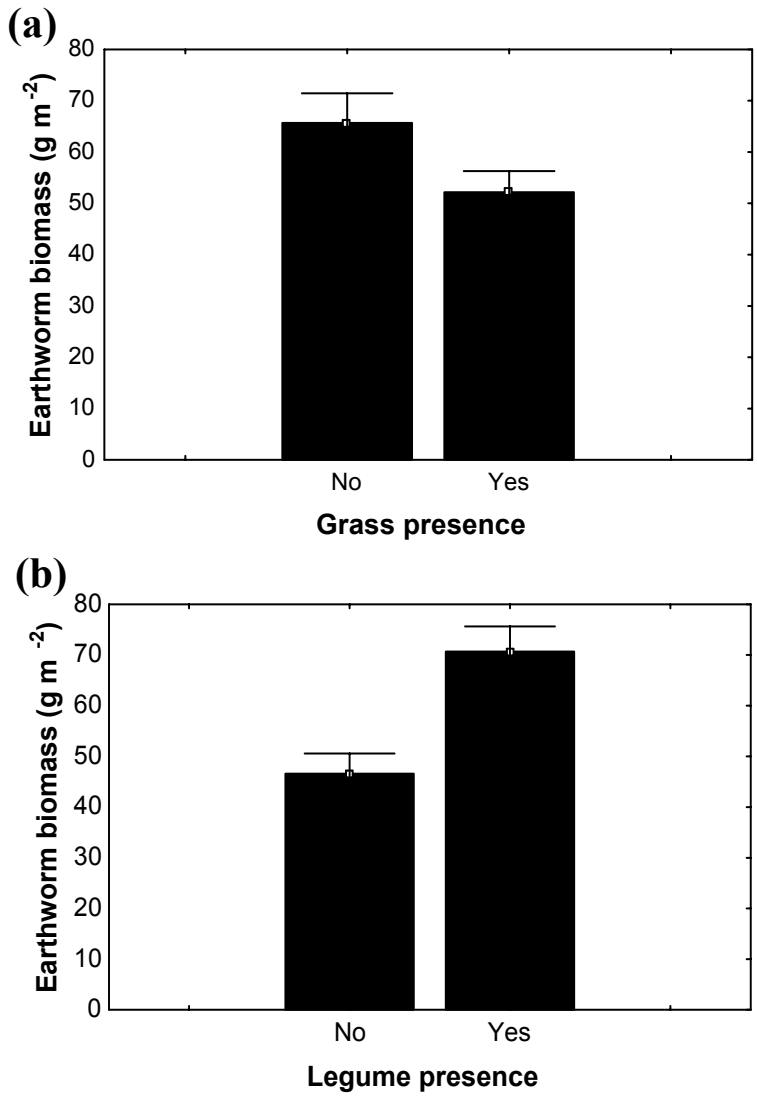

Figure 5.2 Earthworms biomass as affected by (a) presence of grass and (b) presence of legumes. Error bars represent ± SE.

The increase in plant functional group diversity lead only to an increase in total earthworm abundance. Presence of legumes strongly increased total earthworm biomass (+52%) and abundance (+30%), while presence of grasses slightly reduced the total earthworm biomass (-21%) (Fig. 5.2) and abundance (-10%) (Table 5.1).

Analysing the effect of legumes (L) before plant species diversity (S) or functional group diversity (FG) resulted in non-significant diversity effect (S: $F_{2,34}$ = 1.10, P = 0.344 for earthworm biomass and S: $F_{2,34}$ = 1.56, P = 0.224 for earthworm density) suggesting that the diversity effect was due to the presence of legumes. Including root biomass, shoot biomass or total biomass from 2004 as covariables did not affect the diversity effect. Individual species of earthworms were little responsive to changes in plant species and functional group diversity (marginal linear increase with increasing plant species diversity for *A. chlorotica* ($F_{1,34}$ = 2.71, P = 0.109) and *L. terrestris* biomass ($F_{1,34}$ = 2.19, P = 0.148) and density ($F_{1,34}$ = 2.45, P = 0.126)). The identity of plant functional groups only affected *L. terrestris* biomass which was increased in presence of legumes (+58%) and decreased in presence of grasses (-26%)(Table 5.1).

Microbial respiration and biomass

In May 2003, one year after the establishment of the plant communities, no significant effect of plant species and functional group diversity on microbial respiration and biomass was observed, with the exception of a tendency of increased microbial basal respiration in the presence of legumes (Table 5.3). In October 2004 the microbial community responded to experimental manipulations. A very strong block effect was found, with microbial biomass being significantly lower in block 1 (552 µg C_{mic} g^{-1} soil dw) compared to block 2, 3, and 4 (705, 734, 694 µg C_{mic} g^{-1} soil dw, respectively). Microbial respiration showed a different pattern, with a maximum in the block 3 (3.8 µg O_2 h^{-1} g soil dw^{-1}) and a minimum in block 4 (3.4 µg O_2 h^{-1} g soil dw^{-1}), with block 1 (3.6 µg O_2 h^{-1} g soil dw^{-}) and 2 (3.6 µg O_2 h^{-1} g soil dw^{-}) beeing intermediate (Table 5.4). Microbial basal respiration linearly increased with increase in plant species diversity (from 3.2 µg O_2 h^{-1} g soil dw^{-1} in bare ground to 4.2 µg O_2 h^{-1} g soil dw^{-1}in the 16 species treatment), the same trend occurred for microbial biomass (from 587 µg C_{mic} g^{-1} soil dw in bare ground to 715 µg C_{mic} g^{-1} soil dw in the 16 species treatment) (Fig 5.3). No effect of plant functional group diversity was found (Table 5.2). Surprisingly, the presence of legumes increased (+12%) and the presence of grasses slightly

decreased (–5%) microbial biomass, whereas the response of microbial basal respiration was only marginally significant (Table 5.3).

The increase in earthworm density resulted in a slight reduction of microbial respiration (–6%) ($F_{1,38}$ = 4.40, P = 0.040), while microbial biomass was not affected ($F_{1,38}$ = 0.01, P = 0.983). There was no significant difference in microbial biomass and respiration between control and insecticide treatments ($F_{1,86}$ = 0.05, P = 0.824).

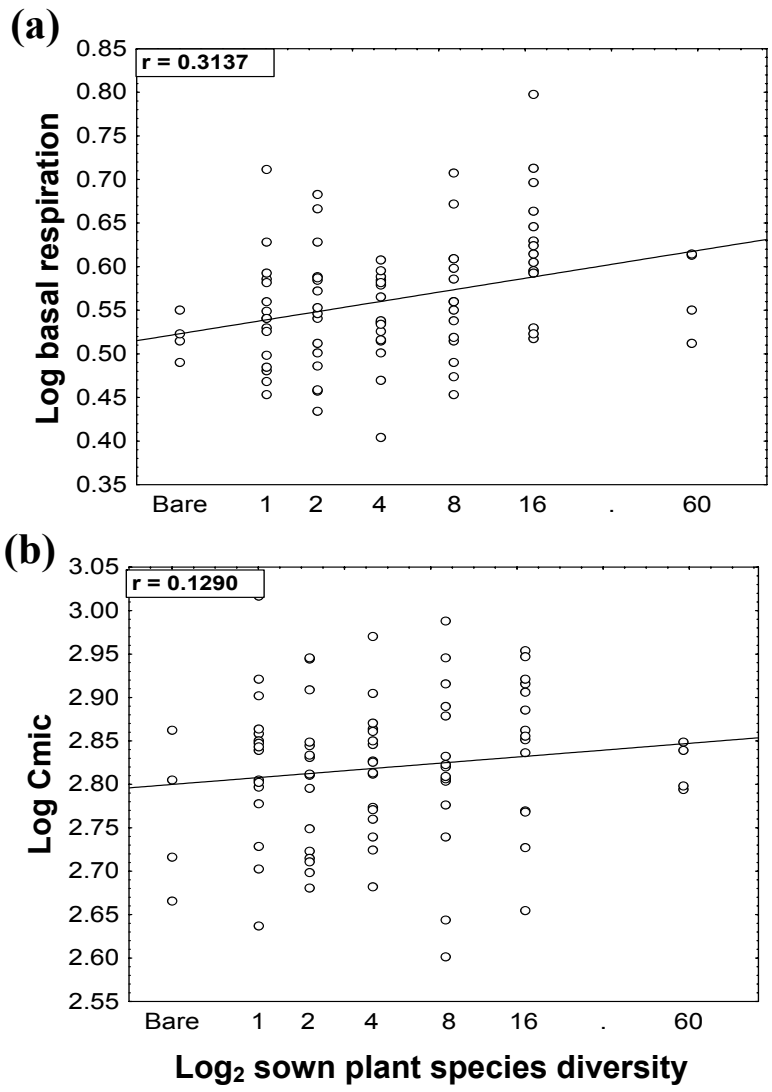

Figure 5.3 Microbial activity (a) and biomass (b) in 2004 as affected by plant species diversity.

Table 5.2 ANOVA table of F-values on the effect of block, functional group diversity(FG), species diversity (S) and presence/absence of legumes (L), grasses (G), small herbs (Sh), tall herbs (Th) on the basal respiration (BR) and microbial biomass (SIR) in April 2003 and October 2004

Treatment factors	BR April 2003	SIR April 2003	BR October 2004	SIR October 2004
Block	$F_{1/69} = 0.62$	$F_{1/69} = 1.38$	**$F_{1/69} = 2.78$***	**$F_{1/69} = 16.20$****
FG	$F_{4/69} = 0.67$	$F_{4/69} = 1.27$	$F_{4/69} = 0.86$	$F_{4/69} = 1.93$
FG log linear	$F_{1/69} = 1.83$	$F_{1/69} = 2.34$	$F_{1/69} = 2.15$	$F_{1/69} = 3.07^{+}$
FG deviation	$F_{3/69} = 0.28$	$F_{1/69} = 0.91$	$F_{1/69} = 0.43$	$F_{1/69} = 1.55$
S	$F_{6/69} = 1.67$	$F_{6/69} = 1.66$	**$F_{6/69} = 2.94$***	$F_{6/69} = 1.22$
S log linear	$F_{1/69} = 0.40$	$F_{1/69} = 0.30$	**$F_{1/69} = 9.60$****	$F_{1/69} = 2.36$
S deviation	$F_{5/69} = 1.58$	$F_{5/69} = 1.94^{+}$	$F_{5/69} = 1.61$	$F_{5/69} = 0.99$
L	$F_{1/69} = 3.04^{+}$	$F_{1/69} = 0.00$	$F_{1/69} = 0.47$	**$F_{1/69} = 7.44$****
G	$F_{1/69} = 1.72$	$F_{1/69} = 0.02$	$F_{1/69} = 3.68^{+}$	**$F_{1/69} = 13.54$*****
Sh	$F_{1/69} = 0.00$	$F_{1/69} = 0.91$	$F_{1/69} = 0.99$	$F_{1/69} = 0.47$
Th	$F_{1/69} = 1.29$	$F_{1/69} = 0.02$	$F_{1/69} = 0.14$	$F_{1/69} = 0.52$

*****, P < 0.001; **, P < 0.01; *, P < 0.05; $^{+}$, P < 0.10**

Litter decomposition

The amount of litter remaining was strongly affected by the block with high amounts of litter remaining in block 1 (1.05 g) and lower amounts remaining in blocks more distant to Saale, block 2 (0.89 g), block 3 (0.87 g) and block 4 (0.66 g), respectively. The responses of individual litter types followed the same pattern with the exception of grass litter which differed only between block 2 (1.0 g) and block 3 (1.4 g). The increase in earthworm density weakly but significantly affected the amount of litter remaining; less litter (27%) remained in plots with increased earthworm density compared to the control (30%) (Table 5.3, Fig. 5.4a). In plots with grasses the amount of litter remaining was marginally significantly increased from 28% to 30% while in the plots with herbs it was slightly decreased from 32% to 28%.

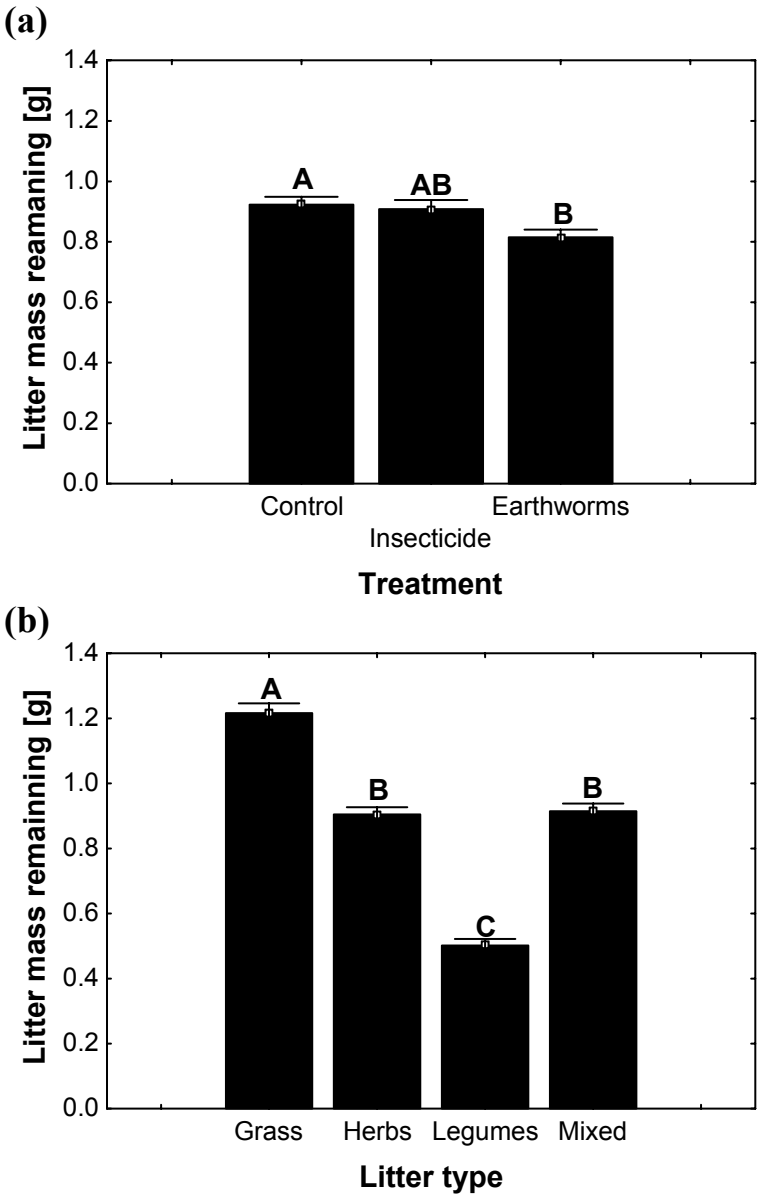

Figure 5.4 Amount of litter remaining in litter bags exposed in the field for 4 months as affected by decomposer treatments (a) and litter type (b). Different letters represent treatments which are significantlly different. Error bars represent ± SE.

Litter decomposition strongly depended on litter type, with legume litter being decomposed fastest (17% remaining) and grasses being slowest (39% remaining), with herbs and mixed litter being intermediate (30% remaining) (Fig. 5.4b). Plant species and functional group diversity did not significantly affect litter decomposition (Table 5.3), but the interaction of

litter type and plant species diversity (litter type x S) suggests that different litter types differentially responded to plant diversity (Table 5.3, Fig. 5.5b)

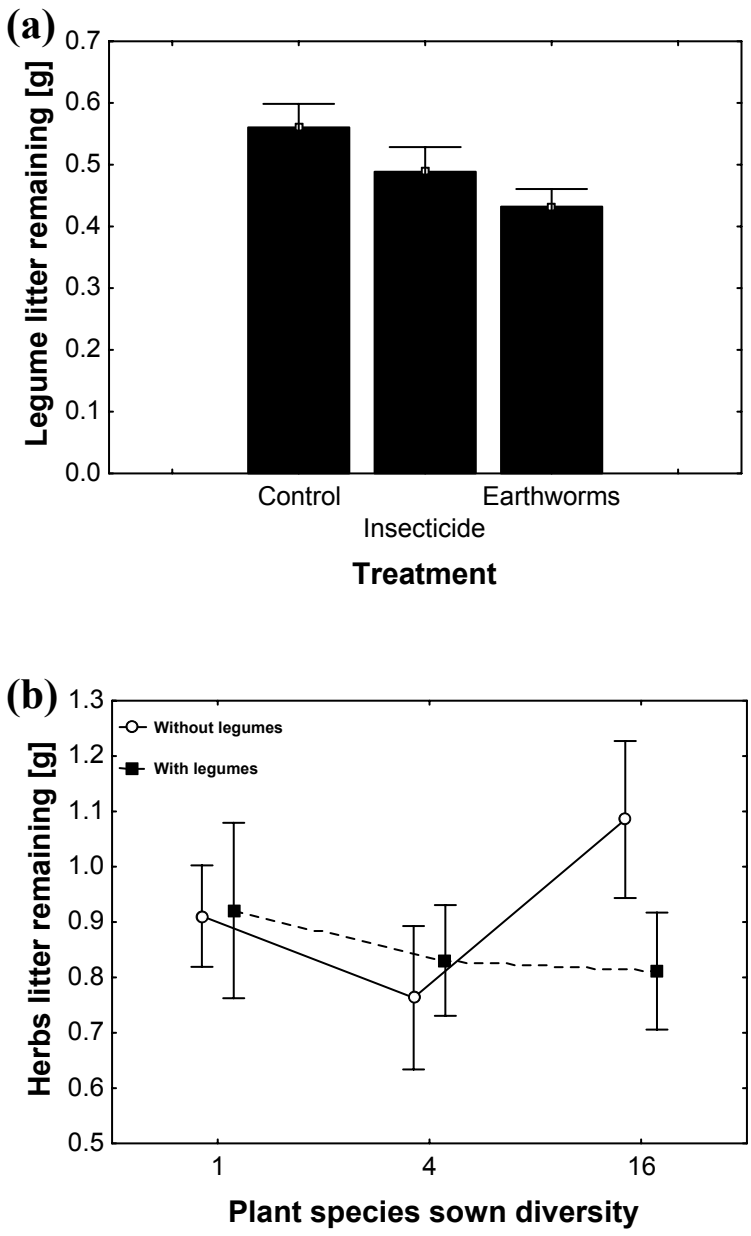

Figure 5.5 (a) Amount of litter remaining in litter bags exposed in the field for 4 months as affected by manipulations in decomposer densities. (b) Herbs litter as affected by the presence of legumes and plant species diversity. Error bars represent ± SE.

Table 5.3 Split-plot ANOVA table of F-values on the effect of block, functional group diversity (FG), species diversity (S) and presence/absence of legumes (L), grasses(G), herbs (H) and the interactions on average amount of litter remaining (means of Gl, Hl, Ll, Mixed) as well as the litter remaining of the individual functional groups of litter (grass litter (Gl), herbs litter (Hl) and legume litter (Ll).

Treatment factors	Litter mass remaining	Gl	Hl	Ll
Block	**$F_{3/29}$ = 7.6*** **	**$F_{3/29}$ = 3.7***	**$F_{3/29}$ = 14.4*** **	**$F_{3/29}$ = 18.9*** **
FG	$F_{3/29}$ = 0.8	$F_{3/29}$ = 0.1	$F_{3/29}$ = 0.4	$F_{3/29}$ = 2.9^{+}
FG log linear	$F_{1/29}$ = 0.9	$F_{1/29}$ = 0.1	$F_{1/29}$ = 0.1	**$F_{1/29}$ = 5.1***
FG deviation	$F_{1/29}$ = 0.7	$F_{1/29}$ = 0.1	$F_{1/29}$ = 0.5	$F_{1/29}$ = 1.8
S	$F_{2/29}$ = 0.9	$F_{2/529}$ = 1.4	$F_{2/529}$ = 0.6	$F_{2/529}$ = 2.4
S log linear	$F_{1/29}$ = 1.7	$F_{1/29}$ = 0.1	$F_{1/29}$ = 0.1	$F_{1/29}$ = 2.1
S deviation	$F_{1/29}$ = 0.1	$F_{1/29}$ = 0.8	$F_{1/29}$ = 0.1	$F_{1/29}$ = 2.8
G	$F_{1/29}$ = 3.9^{+}	$F_{1/29}$ = 3.6^{+}	$F_{1/29}$ = 1.2	$F_{1/29}$ = 2.4
H	$F_{1/29}$ = 3.3^{+}	$F_{1/29}$ = 2.4	$F_{1/29}$ = 0.9	$F_{1/29}$ = 2.0
L	$F_{1/29}$ = 1.7	$F_{1/29}$ = 0.4	$F_{1/29}$ = 0.5	$F_{1/29}$ = 0.5
S x G	$F_{2/29}$ = 1.2	$F_{2/29}$ = 1.6	$F_{2/29}$ = 0.4	$F_{2/29}$ = 2.6^{+}
S x H	$F_{2/29}$ = 0.7	$F_{2/29}$ = 1.5	$F_{2/29}$ = 0.1	$F_{2/29}$ = 2.1
S x L	$F_{2/29}$ = 2.3	$F_{2/29}$ = 1.1	**$F_{2/29}$ = 3.8***	$F_{2/29}$ = 0.5
plotcode	***$F_{29/384}$ = 4.9**** **	***$F_{29/80}$ = 3.2**** **	***$F_{29/80}$ = 2.3**** *	***$F_{29/80}$ = 2.2**** *
Treat	**$F_{2/80}$ = 6.1*** *	$F_{2/80}$ = 2.0	**$F_{2/80}$ = 3.7***	**$F_{2/80}$ = 6.7*** *
Treat x FG	$F_{2/80}$ = 0.6	$F_{2/80}$ = 0.5	$F_{2/80}$ = 1.3	$F_{2/80}$ = 1.8
Treat x S	$F_{2/80}$ = 0.2	$F_{2/80}$ = 1.9	$F_{2/80}$ = 0.2	$F_{2/80}$ = 0.2
Plotcode x treat	*$F_{80/384}$ = 1.5** *	-	-	-
Litter type	**$F_{3/384}$ = 217.9*** **	-	-	-
Litter type x Treat	$F_{6/384}$ = 0.6	-	-	-
Litter type x FG	$F_{9/384}$ = 1.1	-	-	-
Litter type x S	**$F_{6/384}$ = 2.5***	-	-	-

*****, P < 0.001; **, P < 0.01; *, P < 0.05; $^{+}$, P < 0.10**

In plots with grasses the amount of grass litter remaining was increased from 37% to 42%, while the decomposition of herb and legume litter was not significantly affected by the presence of herbs and legumes in the plots. Legume litter was affected by the increased earthworm density (14% remaining compared with 18% remaining in the control) (Table 5.3, Fig 5.5a). Also herb litter decomposition was increased by increased density of earthworms (28% remaining compared with 31% remaining in the control).

Additionally, functional group diversity but not plant species diversity affected the amount of legume litter remaining. In plots with only one plant functional group larger amounts of litter remained (19%) compared to mixtures containing two (14%), three (14%) or four (16%) plant functional groups. On the other side herb litter decomposition was affected by the interaction between plant species diversity and legume presence (S x L) (Fig. 5.5b), with the amount of litter remaining being higher in the absence of legume (36%) compared to mixtures with legumes (27%) in plots with 16 plant species.

5.5 Discussion

Earthworms

The decrease in earthworm biomass and density from block 1 to 4 can be explained through the amount of sand in the soil which decreases from 45% in the block 1 to around 5% in block 4 and through the increase of silt and clay from 43% and 16% to 65% and 23%, respectivelly (Kreutziger 2005). Schulmann & Tiunov (1999) and Marhan (2004) showed that *L. terrestris* benefits form the presence of sand, which facilitates the grinding of organic matter during the gut passage through earthworms. *A. chlorotica* was negatively affected by the increasing content of silt and clay with distance from the Saale river, decreasing in biomass from 6.0 g m^{-2} to 0.5g m^{-2}. It has been documented previously that heavy texture soils sustain lower populations of earthworms then light textured soils (Guild 1948, 1951).

Earthworm biomass and density has been found to increase with plant species richness (Zaller & Arnone 1999, Spehn et al. 2000); however, other authors reported either an idiosyncratic response or no response (Wardle et al. 1999, Gastine et al. 2003, Hedlund et al. 2003). An increase in plant species diversity is associated with an increase in plant biomass production (Hector et al. 1999, Tilman et al. 2001, Spehn et al. 2005, Roscher et al. 2005). Since earthworms are considered to be limited by the amount of plant detritus entering the soil

subsystem (Edwards & Bohlen 1996), earthworm populations likely are affected by changes in plant species diversity via increased plant production and associated increase in the amount of detritus entering into the soil. The increase in earthworm biomass and density with increasing plant species diversity was mainly due to the presence of legumes. Including root, shoot or total biomass as covariables did not reduce the plant diversity effect on earthworm biomass or density suggesting that the effect was not due to increased biomass production. From all five earthworm species only the biomass of the large litter feeding *L. terrestris* was increased in the presence of legumes, and from the endogeic species only the biomass of the *A. chlorotica* was increased marginally (F=3.08, P=0.088) in the presence of legumes. This suggests that different functional groups of earthworms differentially responded to the presence of legumes. In contrast to the anecic litter feeding earthworm species which benefit from litter with low C:N ratio, such as legume litter, endogeic earthworm species are not limited by nitrogen, but by carbon (Scheu & Schaefer 1998, Tiunov & Scheu 2004). The increase in earthworm biomass and density with increasing plant species diversity mainly resulted from legumes which beneficially affected the the biomass of *L. terrestris* and less also that of *A. chlorotica* (F = 2.71 P= 0.109).

Microorganisms

Results of the present study suggest that two years after the establishment of the plant communities, plant species diversity but not plant functional group diversity had a positive influence on the microbial respiration. Microbial biomass and respiration is generally limited by the input of labile carbon (Mikola & Setälä 1998, Joergensen & Scheu 1999) or fresh organic carbon (Schmidt & Paul 1990) and hence plant-biomass production (Zak et al. 1994). Previous studies found microbial biomass to increase with plant species diversity (Spen et al. 2000, Stephan et al. 2000, Zak et al. 2003) but in other studies no significant relationships was found (Wardle et al. 1999, Gastine et al. 2003).

Including root, shoot or total biomass production of 2004 or presence of legumes as covariables in the model did not affect the linear increase of microbial respiration in 2004 with increasing plant species diversity. The increase in microbial respiration with increasing plant species richness could not be explained via effects on plant biomass or presence of legumes. Spehn et al. (2004) explained the increase in catabolic activity of microorganisms through more heterogeneous resources (litter, rhizodeposits, and exudates) or more

heterogeneous soil microhabitats. The increase in earthworm density with increasing plant species diversity supports the conclusion that microhabitat heterogeneity increases microbial respiration.

High density of litter feeding earthworms reduced microbial respiration. It has been found previously that *L. terrestris* feeds predominantly on litter colonized by fungi and that they can compete with microorganisms for resources (Wolter and Scheu 1999, Scheu and Schaefer 1998, Tiunov and Scheu 2004).

The block effect on microbial biomass can be explained by the increase in clay content in block 2, 3 and 4 which is known to correlate with microbial biomass (Muller & Hoper 2004).

Litter

Increasing plant species diversity may affect decomposition rates by (1) changes in quality and quantity of the litter that enters the decomposer subsystem, and (2) changes in microclimate due to more dense vegetation in more diverse plant communities. Since decomposer performance is beneficially affected by litter quality and quantity, therefore changes in plant diversity are accompanied by changes in decomposer communities.

Despite these clear interrelationships, the contribution of macroinvertebrates to litter decomposition has been widely neglected in diversity experiments. In most of the litter decomposition studies performed in diversity experiments until today, the mesh size of the litterbags was too small to allow access of large decomposer invertebrates like earthworms (Wardle et al. 1997, Bardget & Shine 1999, Hector et al. 2000, Knops et al. 2001). Our study suggests that litter feeding and burying by decomposers affects litter decomposition. Macro-decomposers appear to selectively pick the litter of the legumes rich in nitrogen. We observed an increase in earthworm densities and microbial respiration with increasing plant species and functional group diversity. With time is likely to lead to fast burial of nitrogen rich organic material and to faster turnover rates of nitrogen.

An increase in the accumulation of litter rich in nitrogen with increasing productivity as hypothesised by Knops et al. (2001), therefore is unlikely to occur if large litter feeding and burying invertebrates are present.

Rates of litter decomposition were significantly affected by the initial nitrogen concentrations. Increasing plant species and functional group diversity and associated increase in the diversity of litter resources lead to positive additive effects. Contrary to most of the experiments on

litter species richness (Seasted 1984, Salamanca et al. 1998, Smith & Bradford 2003) which found positive or negative non-additive effects in the present experiment effects of functional group litter were additive. Decomposition of mixed litter containing 9 plant species and 3 plant functional groups therefore could have been predicted from the decomposition of single litter functional groups.

The very strong block effect, which presumably resulted from differences in the soil silt and clay content, suggest that processes like decomposition and mineralization are tightly coupled with soil abiotic conditions, rather than with plant diversity. Higher silt and clay content in soil is known to improve the water holding capacity (Kutilek & Nielsen 1994). Soil moisture affects microbial activity (Gulledge & Schimel 1998) as well as earthworm activity (Gerard 1967, Nordstrom 1975) and ultimately litter decomposition (Swift et al. 1979, Cortez 1998).

In conclusion, abiotic conditions, such us soil structure and humidity gradient, were the driving forces of litter decomposition. The fact that after two years from the establishment of the experiment only the legume litter responded to variations in plant functional group diversity suggests that at this stage the role of the plant and functional group diversity for litter decomposition is of little importance. Effects of species or functional group diversity on litter decomposition may have been delayed due to long lasting agricultural land use. However, the decomposer communities (earthworms and microorganisms) were beneficially affected by the increase in plant species and functional group diversity. Earthworms proved to strongly contribute to the decomposition of nitrogen rich organic matter and nitrogen mineralization and their performance depended on plant species diversity and on functional group identity of the plants.

Chapter 6
Final discussion

6.1 Plant diversity effects on earthworms

The amount and quality of plant residues entering the soil are considered to strongly influence earthworms (Edwards & Bohlen 1996). Since increasing plant species and functional group diversity is accompanied by an increase in biomass production (Hector et al. 1999, Tilman et al. 2001, Hooper et al. 2005, Roscher et al. 2005), in turn this is expected to affect earthworm density. The few studies that investigated this issue indeed found an increase of earthworm density with increasing plant species richness (Spehn et al. 2000, Zaller & Arnone 1999); however in other studies an idiosyncratic response or no response was observed (Wardle et al. 1999, Gastine et al. 2003, Hedlund et al. 2003). Only one study tried to differentiate the contribution of above- and belowground plant productivity on the performance of different earthworm functional groups (Spehn et al. 2000).

Our results show that plant species and functional group diversity strongly affected earthworm performance. This was the case in the greenhouse experiment (Chapter 2) as well as in the field (Chapter 5). In the greenhouse the performance of the endogeic earthworm *Aporrectodea caliginosa* (biomass and ^{15}N incorporation) improved with increasing plant species and functional group diversity and depended also on the presence of Collembola. In contrast the anecic *Lumbricus terrestris* was more affected by the presence of legumes. Since the diversity effects on the performance of *A. caliginosa* could neither be explained by plant biomass production nor by the presence of legumes, we conclude that they were due to belowground carbon inputs (rhizodeposits and exudates).

The results from the field experiment are in line with the greenhouse results for *L. terrestris*. Both underline the importance of legumes for the performance of *L. terrestris*. There is evidence that *L. terrestris* can distinguish between different types of litter and prefers litter with low C:N ratio and low concentrations of polyphenols (Satchel 1967, Hendriksen 1990). In contrast, the response of endogeic earthworms was not uniform. One species (*Allolobophora chlorotica*) responded to legume presence and increased biomass with increasing plant species diversity and presence of legumes. In contrast, the other endogeic

species (*Aporectodea caliginosa*, *A. rosea*) were not limited by nitrogen, which is in agreement with Tiunov & Scheu (2004). Results from the greenhouse experiment suggest that endogeic earthworms are influenced by the diversity of the belowground carbon inputs (rhizodeposits and exudates). The lack of response of the endogeic earthworm species may have been due to the fact that they feed on older and more humified organic matter (Pierce 1977, Bouché 1977).

In the field shoot, root or total plant biomass did not correlate with earthworm biomass or density (Chapter 5). It is not surprising that aboveground plant productivity did not correlate with earthworm density, since plant biomass in the field is removed twice per year by mowing (Roscher et al. 2004). Diversity effects generally could be explained by increased incidence of legumes with increasing plant species and functional group diversity (sampling effect) (Chapter 5).

6.2 Earthworm effects on plants

Earthworms can modify plant growth via very different mechanisms, such as nutrient mineralization, soil aeration root and root feeding (Scheu 2003, Brown et al. 2004). However, most of the studies on earthworm-plant interactions explained earthworm mediated increases in plant growth by changes in nutrient availability. Increased nutrient availability in earthworm worked soil, mainly results from feeding on resources rich in organic matter and nutrients (Barois et al. 1999, Cortez & Hamed 2001), promoting microbial activity (Haimi et al. 1992, Alphei et al. 1996), production of casts with readily available nutrients (especially N and P) (Edwards 2004) and nitrogen and mucus excretion (Hameed et al. 1994, Whalen et al. 2000).

The present work showed that earthworms increased the mineralization of nitrogen from litter. Increased plant tissue nitrogen content and increased incorporation of ^{15}N form the labelled litter supports this hypothesis (Chapter 3). As a consequence of the increased nutrient availability, plants disproportionately increase shoot biomass rather than root biomass (Klebsch et al. 1995, Wardle 2002). In contrast, if springtails were also present the shoot to root ratio was significantly increased. Presumably, increased competition for nitrogen between earthworms and springtails (Chapter 3) forced the plants to invest more in the root system.

Earthworms beneficially affected the competitive strength of non-legume plants (grasses and herbs), i.e. plants that depend less on soil nitrogen than legumes which is consistent with previous studies (Wurst et al. 2003, Kreutzer et al. 2004). Furthermore, the interaction of earthworms with other decomposer taxa (springtails) varied with the identity of plant functional groups (Chapter 2); in the absence of legume plants and in presence of springtails the concentration of N in earthworm tissue decreased significantly.

The beneficial effects of earthworms on plant performance were distinct in the greenhouse experiment (Chapter 3), but non-existing in the field experiment (Chapter 5). Two years after the establishment of the plant communities in the field we did not find any significant effects of earthworms on plant performance (biomass) or plant community composition (plant species diversity or cover).

Presumably, low earthworm extraction efficiency in the first year (2003) combined with more variable conditions in the field as compared with the laboratory and the short time elapsed since the experiment has been established were responsible for these non significant effects.

Direct effects of earthworms on plants via translocation of seeds has been little investigated (Scheu 2003). It is known that the gut passage of seeds and the conditions in earthworm casts, such as increased water holding capacity and higher N and P availability (James 1991, Blanchart et al. 1999), affects seed dormancy and seed germination (Grant 1983, Ayanlaya et al. 2001). However, only little is known on how earthworms influence seedling recruitment and ultimately the composition of plant communities (Grant 1983, Pierce et al. 1994, Willems & Huijsmans 1994).

In our laboratory experiment (Chapter 4), earthworms strongly affected seedling recruitment, microhabitat heterogeneity and the soil seedbank. This suggests that earthworms are able to promote but also repress certain plant species depending on seed size and functional group identity of the seeds. Despite the fact that seeds were ingested, seedlings that established in earthworms casts and burrows benefited from beneficial conditions for plant growth in the casts and reduced intra- and interspecific competition due to reduced number of plant seedlings in earthworm treatments. The beneficial effects on seedling recruitment presumably more then compensate for the generally low seed digestion (McRill & Sagar 1973, Grant 1983). In addition, the creation of more heterogeneous microhabitats may promote plant

diversity as suggested by the intermediate disturbance hypothesis (Connell 1978, Fox 1979, Huston 1994) (Chapter 4).

6.3 Plant diversity and decomposition

Decomposition processes are controlled by environmental variables, such as humidity and temperature (Swift et al. 1979). Also, it has been found that plant diversity affects decomposition processes (Salamanca et al. 1998, Bardgett & Shine 1999) via (1) changes in litter species composition that enter the decomposer subsystem; and (2) microclimate changes due to effects of diversity on vegetation structure. Non-additive effects caused by litter diversity are suggested to arise in part from translocation of nutrients or inhibitory litter compounds (Seastedt 1984, Chapmann et al. 1988, Blair et al. 1990, Wardle et al.1977). Changes in microclimate include changes in physical environmental variables like temperature and pH or through changes in the decomposer community (Blair et al. 1990, Wardle et al. 1997).

In our litterbag experiment decomposition and microbial biomass was more strongly affected by soil abiotic conditions than by plant diversity, as suggested by the strong block effects on microbial biomass and litter decomposition (Chapter 5). Both parameters decreased form block 1 to 4. Presumably, this was caused by the gradient in sand content, decreasing from block 1 to 4 and a concomitant increase in clay and silt content. Soil clay and silt content is known to increase the water holding capacity (Kutilek & Nielsen 1994) and microbial biomass (Harris 1981, Muller & Hoper 2004).

Microbial biomass and respiration is considered a sensitive indicator for changes in quality and quantity of organic matter inputs (Van Veen et al.1989, Zak et al. 1994). Microbial activity decreased linearly with increasing plant species and functional group diversity in the greenhouse experiment (Chapter 2), whereas it increased in the field in April 2004 2 years after the establishment of the plant communities (Chapter 4). The latter is in line with results of the studies of Stephan et al. (2000) and Spehn et al. (2000). Microbial activity was correlated with root biomass in both our laboratory and field experiment. In the greenhouse experiment root biomass decreased with increasing plant species and functional group diversity, while it increased in the field. Spehn et al. (2004) explained the increase in catabolic activity of microorganisms in more diverse plant communities by more heterogeneous

resources (litter, rhizodeposits, and exudates) or more heterogeneous soil microhabitats. The increase of earthworm densities with increasing plant species diversity supports the contribution of microhabitat heterogeneity for microbial respiration.

In the litter decomposition experiment (Chapter 5) neither plant species nor plant functional group diversity affected the mass of litter remaining after 4 months. However decomposition/disappearance of nitrogen rich litter was accelerated by macrofauna, especially earthworms. Furthermore, the decomposition of legume litter depended on functional group diversity of the plant mixtures. Since the legume litter decomposed faster with increasing plant functional group diversity, the increased density of earthworms and microbial activity in the more diverse plant community likely contributed to faster turnover of nitrogen. This contradicts the hypothesis of Knops et al. (2001) that litter decomposition does not change with increasing plant species richness leading to an increased nitrogen pool in standing litter. The results from Knops et al. (2001) are based on litterbags of 0.1 mm mesh which excluded the effects of macrofauna on litter decomposition, leading to unrealistic estimates of litter decomposition rates.

The effect of plant functional group diversity on the decomposition of legume litter presumably was due to changes in microenvironment associated with increased earthworm density and biomass with increasing plant diversity. Positive response of earthworm community to an increase in plant species diversity has been also documented by Zaller & Arnone (1999) and Spehn et al. (2000).

In the greenhouse experiment litter decomposition also increased with increasing plant species and functional group diversity and this was accompanied by a higher assimilation of nitrogen out of ^{15}N labeled litter and incorporation into earthworm and plant tissue (Chapter 2 and 3).

Despite the fact that the diversity effects on the heterotrophic activity were less pronounced than the effects due to different soil abiotic conditions, still the increase in plant species and functional group diversity caused a significant increase in the heterotrophic activity, especially of litter rich in nitrogen. This increased activity presumably allowed processing of the additional litter material that enters the decomposer subsystem as a consequence of the increased primary productivity in more diverse plant communities.

In conclusion, the results show that plant diversity affects belowground processes and ecosystem functioning. Legumes represent a key plant functional group influencing decomposition processes via beneficial effects on soil detritivores. Only the increase in

microbial respiration and biomass with increasing plant species diversity could not be explained by the presence of legumes, whereas the performance of earthworms and the decomposition of litter were intimately linked to the presence of legumes (sampling effect).

6.4 Prospects

Most of the studies that investigated the effects of plant diversity on decomposer performance and ecosystem functioning lasted only for months or few years. The soil fauna responds slowly to changes in plant community complexity. However, in the long-term changes in litter quality and quantity, greater diversity of carbon sources that enter the soil (litter and root exudates), and better utilisation of nitrogen resources in soil, will affect the belowground food webs.

Inconsistent results therefore may have resulted from the fact that soil food webs, unlike aboveground food webs, are controlled by long-term effects of litter input and that shifts in plant species composition may manifest over longer time scales only. Knowing the complexity of the interactions between below- and aboveground communities as well as the fact that recently established communities need time to reach steady state condition there is a need for long-term biodiversity studies. A better understanding of the factors by which changes in plant diversity affect the belowground food web is necessary. The input of aboveground and belowground litter material (shoot vs. roots and rhizodeposits) need to be clarified, as well as their effect on decomposer community composition and functioning.

Little attention has been paid to investigate the resistance and resilience of the soil food web and their heterotrophic performance following disturbances, such as pollution and drought. Another interesting and unexplored aspect is the invasibility of plant communities as affected by the composition of the soil decomposer community.

Plant community establishment from seeds is strongly affected by translocation of seeds by decomposers, in particular earthworms, and this varies with seed size, plant functional group and microhabitat heterogeneity. More detailed studies are necessary to investigate these relationships in more complex communities where seedlings have to establish and compete in the presence of groving plants.

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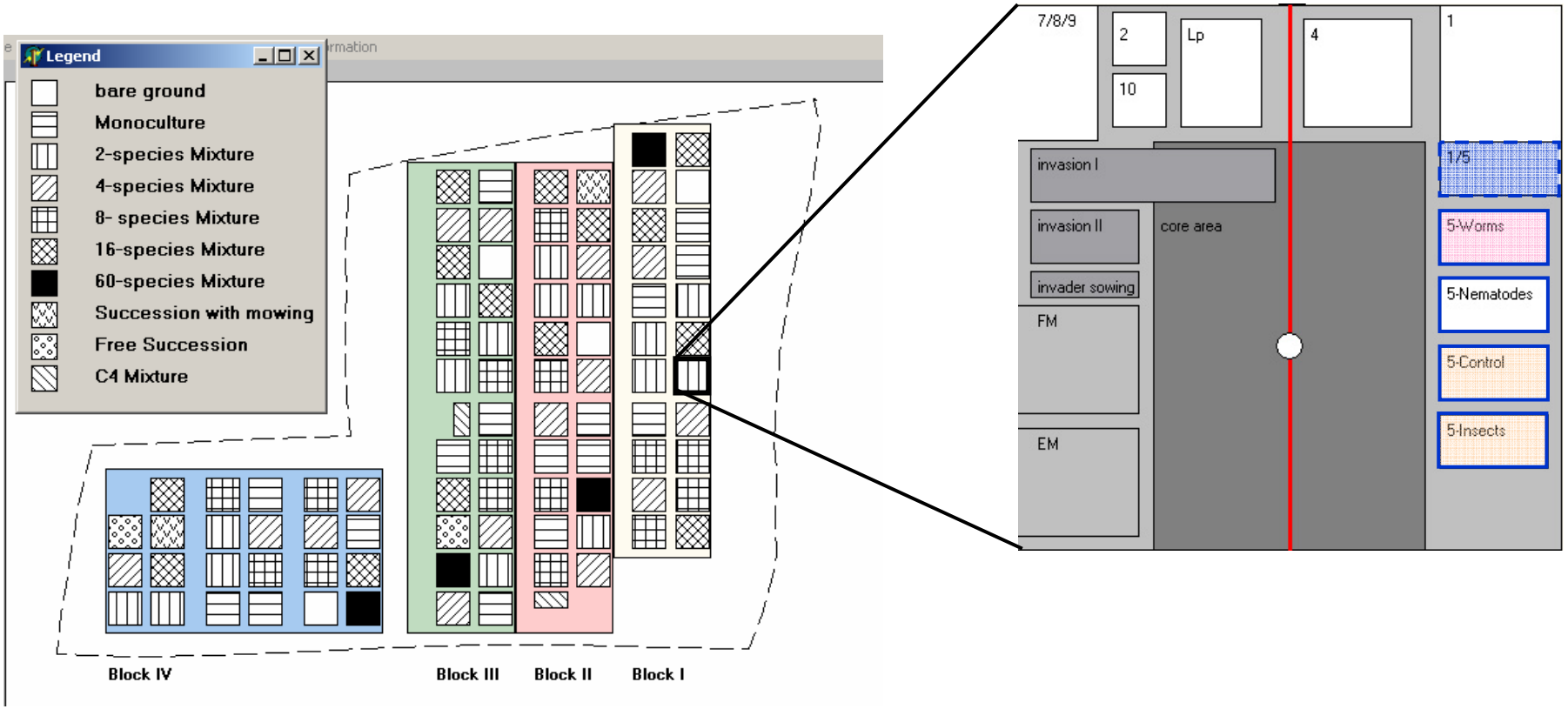
Appendix 1

“The Jena Experiment” Design.



Appendix 2

Plant species comoposition form the Jena experiment

F = number of functional groups

G = number of species corresponding to the functional groups: grasses/ small herbs/ thall herbs/ legumes

Monocultures

F:1; G:1/0/0/0 *Cynosurus cristatus*

F:1; G:1/0/0/0 *Festuca pratensis*

F:1; G:1/0/0/0 *Festuca rubra*

F:1; G:1/0/0/0 *Poa pratensis*

F:1; G:0/1/0/0 *Plantago lanceolata*

F:1; G:0/1/0/0 *Bellis perennis*

F:1; G:0/1/0/0 *Prunella vulgaris*

F:1; G:0/1/0/0 *Veronica chamaedrys*

F:1; G:0/0/1/0 *Cirsium oleraceum*

F:1; G:0/0/1/0 *Geranium pratense*

F:1; G:0/0/1/0 *Crepis biennis*

F:1; G:0/0/1/0 *Galium mollugo*

F:1; G:0/0/0/1 *Lathyrus pratensis*

F:1; G:0/0/0/1 *Vicia sepium*

F:1; G:0/0/0/1 *Onobrychis viciifolia*

F:1; G:0/0/0/1 *Medicago sativa*

2-species-mixtures

F:1; G:2/0/0/0 *Festuca pratensis, Dactylis glomerata*

F:1; G:2/0/0/0 *Festuca rubra, Trisetum flavescens*

F:1; G:0/2/0/0 *Cerastium holosteoides, Taraxacum officinale*

F:1; G:0/2/0/0 *Plantago lanceolata, Bellis perennis*

F:1; G:0/0/2/0 *Heracleum sphondylium, Daucus carota*

F:1; G:0/0/2/0 *Ranunculus acris, Sanguisorba officinalis*

F:1; G:0/0/0/2 *Onobrychis viciifolia, Medicago lupulina*

F:1; G:0/0/0/2 *Lotus corniculatus, Trifolium pratense*

F:2; G:1/1/0/0 *Trisetum flavescens, Taraxacum officinale*

F:2; G:1/1/0/0 *Poa pratensis, Plantago lanceolata*

F:2; G:0/0/1/1 *Ranunculus acris, Trifolium campestre*

F:2; G:0/0/1/1 *Daucus carota, Medicago sativa*

Appendix 2 continued

F:2; G:1/0/1/0 *Festuca pratensis, Carum carvi*

F:2; G:1/0/1/0 *Alopecurus pratensis, Daucus carota*

F:2; G:0/1/0/1 *Ranunculus repens, Medicago lupulina*

F:2; G:0/1/0/1 *Plantago lanceolata, Trifolium dubium*

4-species-mixtures

F:1; G:4/0/0/0 *Alopecurus pratensis, Poa trivialis, Anthoxanthum odoratum, Bromus erectus*

F:1; G:0/4/0/0 *Prunella vulgaris, Primula veris, Plantago lanceolata, Ajuga reptans*

F:1; G:0/0/4/0 *Campanula patula, Geranium pratense, Cardamine pratensis, Knautia arvensis*

F:1; G:0/0/0/4 *Lotus corniculatus, Onobrychis viciifolia, Medicago sativa, Medicago lupulina*

F:2; G:2/2/0/0 *Poa trivialis, Bromus erectus, Plantago lanceolata, Prunella vulgaris*

F:2; G:0/0/2/2 *Cardamine pratensis, Crepis biennis, Medicago lupulina, Vicia sepium*

F:2; G:2/0/2/0 *Festuca pratensis, Luzula campestris, Achillea millefolium, Crepis biennis*

F:2; G:0/2/0/2 *Taraxacum officinale, Plantago lanceolata, Medicago lupulina, Lathyrus pratensis*

F:3; G:2/1/1/0 *Arrhenatherum elatius, Luzula campestris, Prunella vulgaris, Campanula patula*

F:3; G:1/0/2/1 *Trisetum flavescens, Tragopogon pratensis, Heracleum sphondylium, Medicago sativa*

F:3; G:1/1/0/2 *Phleum pratense, Cerastium holosteoides, Trifolium hybridum, Vicia cracca*

F:3; G:0/2/1/1 *Cerastium holosteoides, Leontodon autumnalis, Knautia arvensis, Vicia cracca*

F:4; G:1/1/1:1 *Bromus hordeaceus, Ranunculus repens, Leucanthemum vulgare, Trifolium repens*

F:4; G:1/1/1:1 *Arrhenatherum elatius, Plantago lanceolata, Anthriscus sylvestris, Trifolium campestre*

F:4; G:1/1/1:1 *Anthoxanthum odoratum, Prunella vulgaris, Knautia arvensis, Trifolium pratense*

F:4; G:1/1/1:1 *Festuca pratensis, Plantago lanceolata, Campanula patula, Onobrychis viciifolia*

8-species-mixtures

F:1; G:8/0/0/0 *Holcus lanatus, Cynosurus cristatus, Poa trivialis, Dactylis glomerata, Arrhenatherum elatius, Festuca rubra, Alopecurus pratensis, Trisetum flavescens*

F:1; G:0/8/0/0 *Bellis perennis, Taraxacum officinale, Ajuga reptans, Leontodon autumnalis, Veronica chamaedrys, Glechoma hederacea, Primula veris, Prunella vulgaris*

F:1; G:0/0/8/0 *Knautia arvensis, Sanguisorba officinalis, Geranium pratense, Leucanthemum vulgare, Anthriscus sylvestris, Galium mollugo, Heracleum sphondylium, Ranunculus acris*

F:1; G:0/0/0/8 *Trifolium pratense, Onobrychis viciifolia, Medicago sativa, Trifolium dubium, Lathyrus pratensis, Trifolium hybridum, Medicago lupulina, Trifolium campestre*

F:2; G:4/4/0/0 *Anthoxanthum odoratum, Festuca rubra, Bromus hordeaceus, Avenula pubescens, Plantago lanceolata, Ajuga reptans, Veronica chamaedrys, Taraxacum officinale*

Appendix 2 continued

F:2; G:0/0/4/4 *Cardamine pratensis, Knautia arvensis, Heracleum sphondylium, Campanula patula, Lotus corniculatus, Trifolium campestre, Trifolium repens, Trifolium hybridum*

F:2; G:4/0/4/0 *Phleum pratense, Festuca rubra, Bromus erectus, Alopecurus pratensis, Sanguisorba officinalis, Ranunculus acris, Heracleum sphondylium, Cardamine pratensis*

F:2; G:0/4/0/4 *Cerastium holosteoides, Glechoma hederacea, Taraxacum officinale, Leontodon autumnalis, Trifolium repens, Lathyrus pratensis, Vicia cracca, Trifolium campestre*

F:3; G:2/3/3/0 *Festuca pratensis, Bromus erectus, Primula veris, Cerastium holosteoides, Ajuga reptans, Achillea millefolium, Carum carvi, Pimpinella major*

F:3; G:3/0/2/3 *Anthoxanthum odoratum, Poa trivialis, Bromus erectus, Anthriscus sylvestris, Leucanthemum vulgare, Onobrychis viciifolia, Trifolium hybridum, Lotus corniculatus*

F:3; G:3/3/0/2 *Cynosurus cristatus, Phleum pratense, Trisetum flavescens, Veronica chamaedrys, Glechoma hederacea, Primula veris, Medicago lupulina, Lotus corniculatus*

F:3; G:0/2/3/3 *Cerastium holosteoides, Leontodon hispidus, Crepis biennis, Galium mollugo, Sanguisorba officinalis, Lotus corniculatus, Medicago lupulina, Onobrychis viciifolia*

F:4; G:2/2/2/2 *Luzula campestris, Phleum pratense, Leontodon hispidus, Veronica chamaedrys, Knautia arvensis, Sanguisorba officinalis, Trifolium dubium, Trifolium hybridum*

F:4; G:2/2/2/2 *Phleum pratense, Poa trivialis, Taraxacum officinale, Primula veris, Sanguisorba officinalis, Anthriscus sylvestris, Trifolium repens, Trifolium dubium*

F:4; G:2/2/2/2 *Luzula campestris, Trisetum flavescens, Plantago lanceolata, Leontodon hispidus, Daucus carota, Anthriscus sylvestris, Trifolium campestre, Trifolium repens*

F:4; G:2/2/2/2 *Holcus lanatus, Bromus hordeaceus, Primula veris, Ranunculus repens, Leucanthemum vulgare, Heracleum sphondylium, Medicago lupulina, Onobrychis viciifolia*

16-species-mixtures

F:2; G:8/8/0/0 *Arrhenatherum elatius, Festuca pratensis, Alopecurus pratensis, Bromus erectus, Phleum pratense, Poa pratensis, Anthoxanthum odoratum, Holcus lanatus, Veronica chamaedrys, Ranunculus repens, Prunella vulgaris, Bellis perennis, Leontodon autumnalis, Primula veris, Plantago lanceolata, Leontodon hispidus*

F:2; G:0/0/8/8 *Campanula patula, Centaurea jacea, Geranium pratense, Cirsium oleraceum, Cardamine pratensis, Rumex acetosa, Tragopogon pratensis, Anthriscus sylvestris, Trifolium dubium, Trifolium hybridum, Trifolium campestre, Trifolium repens, Vicia cracca, Medicago sativa, Trifolium pratense, Vicia sepium*

Appendix 2 continued

F:2; G:8/0/8/0 *Poa pratensis, Bromus hordeaceus, Alopecurus pratensis, Poa trivialis, Holcus lanatus, Anthoxanthum odoratum, Trisetum flavescens, Avenula pubescens, Pimpinella major, Anthriscus sylvestris, Geranium pratense, Centaurea jacea, Campanula patula, Leucanthemum vulgare, Achillea millefolium, Heracleum sphondylium*

F:2; G:0/8/0/8 *Prunella vulgaris, Ajuga reptans, Taraxacum officinale, Glechoma hederacea, Veronica chamaedrys, Leontodon hispidus, Cerastium holosteoides, Ranunculus repens, Trifolium campestre, Vicia cracca, Lathyrus pratensis, Trifolium hybridum, Vicia sepium, Onobrychis viciifolia, Medicago lupulina, Trifolium repens*

F:3; G:5/5/6/0 *Phleum pratense, Luzula campestris, Cynosurus cristatus, Arrhenatherum elatius, Poa pratensis, Cerastium holosteoides, Taraxacum officinale, Glechoma hederacea, Leontodon hispidus, Ranunculus repens, Heracleum sphondylium, Galium mollugo, Ranunculus acris, Pastinaca sativa, Anthriscus sylvestris, Knautia arvensis*

F:3; G:5/0/5/6 *Cynosurus cristatus, Festuca pratensis, Trisetum flavescens, Phleum pratense, Poa trivialis, Centaurea jacea, Rumex acetosa, Sanguisorba officinalis, Achillea millefolium, Campanula patula, Trifolium hybridum, Lotus corniculatus, Vicia cracca, Vicia sepium, Lathyrus pratensis, Onobrychis viciifolia*

F:3; G:6/5/0/5 *Festuca pratensis, Bromus hordeaceus, Avenula pubescens, Anthoxanthum odoratum, Poa trivialis, Arrhenatherum elatius, Taraxacum officinale, Ranunculus repens, Ajuga reptans, Prunella vulgaris, Glechoma hederacea, Lotus corniculatus, Trifolium pratense, Vicia sepium, Vicia cracca, Medicago sativa*

F:3; G:0/6/5/5 *Veronica chamaedrys, Leontodon autumnalis, Ajuga reptans, Plantago lanceolata, Bellis perennis, Leontodon hispidus, Ranunculus acris, Sanguisorba officinalis, Achillea millefolium, Geranium pratense, Knautia arvensis, Trifolium hybridum, Medicago sativa, Lotus corniculatus, Vicia sepium, Onobrychis viciifolia*

F:4; G:4/4/4/4 *Cynosurus cristatus, Luzula campestris, Alopecurus pratensis, Bromus hordeaceus, Leontodon autumnalis, Cerastium holosteoides, Veronica chamaedrys, Taraxacum officinale, Crepis biennis, Carum carvi, Pimpinella major, Heracleum sphondylium, Trifolium hybridum, Trifolium campestre, Lathyrus pratensis, Onobrychis viciifolia*

F:4; G:4/4/4/4 *Phleum pratense, Festuca rubra, Anthoxanthum odoratum, Bromus erectus, Ranunculus repens, Ajuga reptans, Bellis perennis, Veronica chamaedrys, Geranium pratense, Crepis biennis, Rumex acetosa, Galium mollugo, Vicia cracca, Onobrychis viciifolia, Trifolium repens, Trifolium dubium*

Appendix 2 continued

F:4; G:4/4/4/4 *Alopecurus pratensis, Bromus hordeaceus, Poa pratensis, Cynosurus cristatus, Ranunculus repens, Cerastium holosteoides, Ajuga reptans, Primula veris, Cardamine pratensis, Geranium pratense, Anthriscus sylvestris, Campanula patula, Medicago lupulina, Vicia sepium, Trifolium dubium, Trifolium campestre*

F:4; G:4/4/4/4 *Avenula pubescens, Poa pratensis, Anthoxanthum odoratum, Bromus hordeaceus,Plantago lanceolata, Taraxacum officinale, Ajuga reptans, Ranunculus repens, Anthriscus sylvestris, Geranium pratense, Tragopogon pratensis, Carum carvi, Trifolium campestre, Vicia cracca, Lathyrus pratensis, Lotus corniculatus*

F:1; G:16/0/0/0 *Anthoxanthum odoratum, Arrhenatherum elatius, Bromus erectus, Cynosurus cristatus, Dactylis glomerata, Festuca pratensis, Phleum pratense, Trisetum flavescens, Alopecurus pratensis, Avenula pubescens, Bromus hordeaceus, Festuca rubra, Holcus lanatus, Luzula campestris, Poa pratensis, Poa trivialis*

F1; G:0/0/16/0 *Pastinaca sativa, Cardamine pratensis, Campanula patula, Heracleum sphondylium, Ranunculus acris, Anthriscus sylvestris, Achillea millefolium, Tragopogon pratensis, Sanguisorba officinalis, Galium mollugo, Crepis biennis, Rumex acetosa, Cirsium oleraceum, Leucanthemum vulgare, Daucus carota, Geranium pratens*e

Appendix 3

Scheme of the 64 plant species mixtures set up in the experiment varying in species identity, number of species and composition of functional groups (Grasses **(G)** Small herbs **(Sh)** Tall herbs **(Th)** and Legumes **(L)**). Four animal treatments were set up per plant species mixture (Control without animals, with Earthworms, with Collembola, with Earthworms and Collembola).

(G): Ao= *Anthoxantum odoratum* L., Ap= *Alopecurus pratensis* L., Be= *Bromus erectus* HUDS., Bh= *Bromus hordeaceus* L., Cc= *Cynosurus cristatus* L., Dg= *Dactylis glomerata* L., Fp= *Festuca pratensis* HUDS., Fr= *Festuca rubra* L., Hl= *Holcus lanatus* L., PHp= *Phleum pratense* L., Pp= *Poa pratensis* L., Pt= *Poa trivialis* L., Tf= *Trisetum flavescens*;

(Sh): Bp= *Bellis perennis* L., Gh= *Glechoma hederacea* L., La= *Leontodon autumnalis* L., Lh= *Leontodon hispidus* L., Pl= *Plantago lancelolata* L., Pm= *Plantago media* L., Pv= *Prunella vulgaris* L., To= *Taraxacum officinale* WEBER, Vc= *Veronica chamaedrys* L.;

(Th): Am= *Achillea millefolium* L., Cb= *Crepis benis* L., Cj= *Centaurea jacea* L., Co= *Cirsium oleraceum* L., Cp= *Cardamine pratensis* L., Dc= *Daucus carota* L., Ga= *Galium mollugo* L., Ka= *Knautia arvensis* L., Lv= *Leucanthemum vulgare* Lam., Ra= *Rumex acetosa* L., TRp= *Tragopogon pratensis* L.

(L): Lp= *Lathyrus pratensis* L., Lc= *Lotus corniculatus* L., Ml= *Medicago lupulina* L., Ms= *Medicago x varia* MARTYN, Ov= *Onobrychis viciifolia* SCOP., Td= *Trifolium dubium* SIBTH., Th= *Trifolium hybridum* L., Tr= *Trifolium repens* L., Tp= *Trifolium pratense* L., VIc= *Vicia cracca* L

Appendix 3 continued

Species mixture	Species diversity	Functional group diversity	Functional group composition	Species
1- 16	1	1	4 G, 4 Sh, 4 Th, 4 L	monoculture of Cc; Fp; Fr; Pp; Pl; Bp; Pv; Gh; Co; Dc; Cb; Gm; Lp; Vic; Ov; Ms
17-18	2	1	2G	Fp, Dg; Fr, Tf
19-20	2	1	2 Sh	Bp, To; Pl, Bp
21-22	2	1	2 Th	Cb, Dc; Cj, Ra
23-24	2	1	2 L	Ov, Tr; Lc, Tp
25	2	2	GSh	Tf, To
26	2	2	GSh	Pp, Pl
27	2	2	ThL	Cj, Td
28	2	2	ThL	Dc, Ms
29	2	2	GTh	Fp, Cj
30	2	2	GTh	Ap, Dc
31	2	2	ShL	La, Tr
32	2	2	ShL	Pl, Td
33	4	1	G	Ap, Pt, Ao, Be
34	4	1	Sh	Pv, Pm, Pl, Lh
35	4	1	Th	Am, Dc, Cp, Ka
36	4	1	L	Lc, Ov, Ms, Tr
37	4	2	GSh	Pt, Be, Pl, Pv
38	4	2	ThL	Cp, Cb, Tr, VIc

Appendix 3 continnued

39	4	2	GTh	Fp, Pt, Am, Cb
40	4	2	ShL	To, Pl, Tr, Lp
41	4	3	GShTh	Ao, Pt, Pv, Am
42	4	3	GThL	Tf, TRp, Cb, Ms
43	4	3	GShL	Php, Bp, Th, VIc
44	4	3	ShThL	Bp, La, Ka, VIc
45	4	4	GShThL	Bh, La, Lv, Ml
46	4	4	GShThL	Ao, Pl, Co, Td
47	4	4	GShThL	Ao, Pv, Ka, Tp
48	4	4	GShThL	Fp, Pl, Am, Ov
49	8	1	G	Hl, Cc, Pt, Dg, Ao, Fr, Ap, Tf
50	8	1	Sh	Bp, To, Lh, La, Gh, Vc, Pm, Pv
51	8	1	Th	Ka, Ra, Dc, Lv, Co, Gm, Cb, Cj,
52	8	1	L	Tp, Ov, Ms, Td, Lp, Th, Tr, Lc
53	8	2	GSh	Ao, Fr, Bh, Cc, Pl, Lh, Gh, To
54	8	2	ThL	Cp, Ka, Cb, Am, Lc, Td, Ml, Th
55	8	2	GTh	Php, Fr, Be, Ap, Ra, Cj, Cb, Cp
56	8	2	ShL	Bp, Vc, To, La, Ml, Lp, VIc, Td
57	8	3	GShTh	Fp, Be, Pm, Pv, Lh, Am, Cj, TRp
58	8	3	GThL	Ao, Pt, Be, Co, Lv, Ov, Th, Lc
59	8	3	GShL	Cc, Php, Tf, Gh, Vc, Pm, Tr, Lc
60	8	3	ShThL	Bp, Lh, Cb, Gm, Ra, Lc, Tr, Ov
61	8	4	GShThL	Pt, Php, Lh, Gh, Ka, Ra, Td, Th
62	8	4	GShThL	Php, Pt, To, Pm, Ra, Co, Ml, Td
63	8	4	GShThL	Pt, Tf, Pl, Lh, Dc, Co, Td, Ml
64	8	4	GShThL	Hl, Bh, Pl, La, Lv, Cb, Tr, Ov

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